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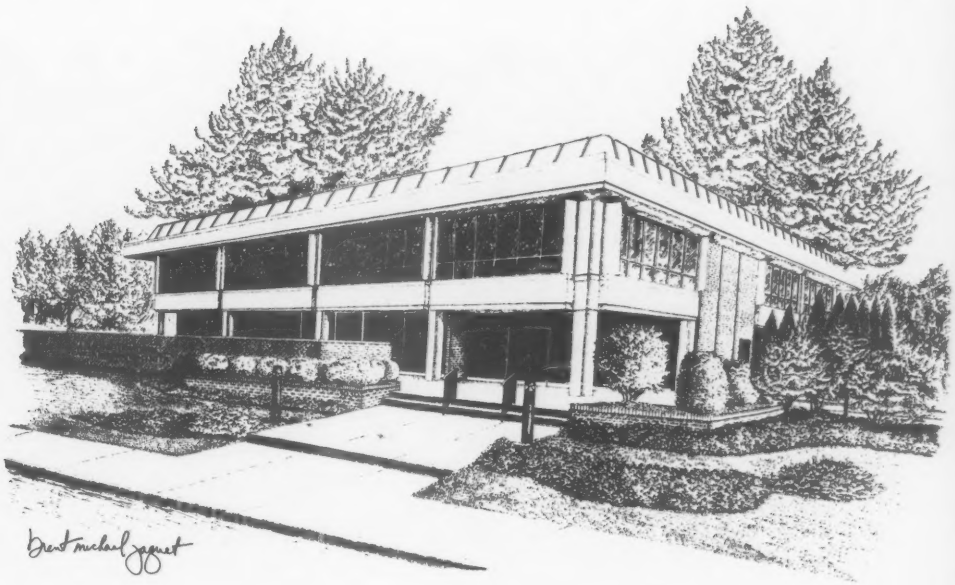

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Surgical Oncology Research Development: The Perspective of the National Cancer Institute[1]

Bruce A. Chabner,* Gregory A. Curt, and Susan Molloy Hubbard[2]

Surgery has a central role in the treatment of cancer. For the almost 800,000 patients who are annually diagnosed as having malignancies, surgery is the primary form of therapy in > 400,000. One-half of these patients will be cured and will need no further treatment. Looking at the same data from a different perspective, of the 350,000 patients who are annually cured of cancer, 60% are cured by surgery, 25% by radiotherapy, and 13% by chemotherapy.

Yet, despite its critical role, the efficacy of surgery alone as a modality for cancer cure has changed little over the past 20 years. This is because the operation, although curative for patients with only localized disease, remains inadequate for the 70% of patients with solid tumors who have obvious or occult metastasis when first diagnosed. It is to these 70% of patients that National Cancer Institute (NCI) research has been directed.

During the past 20 years, the greatest changes and improvements in cancer treatment have occurred because of the development of high-energy radiotherapy and new drugs. High-energy radiotherapy, as developed in the 1950s and applied in the 1960s, allowed the delivery of curative radiation to well-defined fields with tolerable damage to adjacent normal tissues. As a result of this technical development, diseases such as malignant lymphoma, seminoma, head and neck cancer, and prostate cancer are curable when discovered at an early stage. Drugs such as doxorubicin, vinblastine, cisplatin, and bleomycin, all developed during the past two decades, have also had a significant impact on cancer cure and survival and are now regularly used by physicians in practice, including surgeons, to treat patients who would have been considered incurable a short time ago. As a logical sequence of these advances, clinicians from all three disciplines—medical oncology, radiotherapy, and surgery—have come to the realization that the coordinated use of integrated treatment can achieve results unobtainable by single modalities. Adjuvant chemotherapy and multimodality therapy did not exist in the early 1960s, but now are familiar to every practicing physician as the result of studies in breast cancer, sarcomas, lung cancer, and esophageal cancer. Thus, while the actual performance of basic surgical procedures such as colectomies, radical neck dissection, or mastectomy may have changed little over the past 20 years, the results of surgery have been enhanced by the coordinated use of drugs and radiotherapy.

For example, in childhood rhabdomyosarcoma, the most common soft tissue sarcoma of infants and young children, surgery alone resulted in long-term survival of only 10%–20% of patients, with the remainder dying from unresectable local or metastatic disease (1). With the introduction of radiotherapy, approximately one-half of the patients achieved long-term survival. Finally, with the addition of combination chemotherapy, long-term cures in this disease have reached 80%–90% (2). The same improvements with multimodality approaches have been successful in the soft tissue sarcoma and breast cancer.

It is precisely because of the rapid development of medical oncology and radiotherapy in recent years that the demands placed on the oncologic surgeon have increased. Since knowledge has accrued rapidly in the other cancer-related specialties, the surgeon must not only keep current with changes in operative techniques and the surgical literature, but he must also become acquainted with the rapidly changing potentials of these newer fields of cancer treatment. These other fields have influenced every phase of surgical practice, from biopsy to definitive surgery and postoperative care.

Thus, in treating breast cancer, the surgeon must be aware of the need to obtain tissue for estrogen and progesterone receptors during the initial biopsy, must present an honest appraisal of radiotherapy versus mastectomy to the patient, and must be aware of the likely benefits and side effects of adjuvant chemotherapy after primary surgical treatment. He must know the benefits of modern cancer screening techniques, such as mam-

[1]Received Mar 9, 1984; accepted Mar 15, 1984.

[2]Division of Cancer Treatment (*B. A. Chabner* and *G. A. Curt*) and Scientific Information Branch, Office of International Affairs (*S. M. Hubbard*), National Cancer Institute, Bethesda, MD.

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mography, and the potential of reconstructive surgery. Thus, the surgeon's own responsibilities run the gamut from prevention to diagnosis, treatment, and rehabilitation, but he must also have knowledge of the treatment potentials of medical and radiotherapy colleagues. The growing complexity of the surgeon's task has clearly created the need for specialized training programs in surgical oncology.

While these important changes have taken place in the practice of cancer surgery, equally significant changes have occurred in clinical and basic cancer research over the past two decades. Modern techniques of randomization and statistical analysis have revolutionized the approach to large-scale therapeutic trials. The application of these methods to cancer research has required the treatment and monitoring of large numbers of patients. This has led to multi-institution trials supported by statistical centers, an approach incorporated by surgically oriented groups in the areas of breast, prostate, bladder, gastrointestinal, and lung cancers. Thus, clinical trials research in surgical oncology has shifted from largely retrospective single-institution studies to cooperative group studies.

Second, in laboratory research, the advances in cell biology, biochemistry, and molecular biology have greatly increased the competition facing clinical researchers in the search for R01 and P01 grants. Without several years of dedicated laboratory training, these physicians simply cannot successfully compete for funding. The medical oncologists have fared well in this transition because the better training programs have strong laboratory components, which have been traditional for departments of medicine. However, the lack of cancer research opportunities in most surgical departments has significantly hampered young surgeons who have to establish academic careers in this field, where the most exciting efforts are biochemical and molecular in their focus.

Recognizing both the needs and potentials of surgical oncology, the Division of Cancer Treatment (DCT) at the NCI has strongly supported treatment research in this area. These initiatives have included efforts to increase effectiveness of surgical oncology teaching at medical schools; initiation of new, targeted training grants for young surgeons, planning grants for establishing surgical research efforts; program announcements to encourage submission of surgical oncology research grants by established investigators; a more active role by DCT in assisting applicants to revise grants and track their progress; and the recommendation of appropriate individuals for service on review committees. The following report summarizes the history of DCT support of surgical oncology and reviews our current efforts and plans.

History of DCT Support of Surgical Oncology

DCT activities in surgical oncology began in 1976 when the Division accepted responsibility for both surgical and radiation oncology in its intramural and extramural programs. Prior to that time, surgical oncology had been located with radiotherapy in the Division of Cancer Biology and Diagnosis. To evaluate the status of efforts in these two specialties and to identify needs, the Board of Scientific Counselors of DCT established two subcommittees, the Radiation Oncology Subcommittee and the Surgical Oncology Subcommittee, each composed entirely of Board members. The first report of the Surgical Oncology Subcommittee was made to the Board by Dr. Bernard Fisher in October 1976. The Subcommittee focused primarily on the need for increased support for surgical oncology research and presented to the Board several recommendations for stimulating research in this area. These included the establishment of a separate study section to review surgical oncology grants and the inclusion of additional surgical representatives on NCI councils and peer review committees reviewing grants, and the initiation of a Surgical Evaluation Program within DCT.

The Division responded to each of these suggestions (table 1). A Surgical Oncology Section was established in 1978 within the Cancer Therapy Evaluation Program in DCT to serve as a focus for surgical oncology research within the Division. In addition, funds were set aside for a Request for Application (RFA) for research grants entitled "New Approaches in Surgical Oncology." The first RFA was issued in 1978 with $1.6 million in set-aside

TABLE 1.—Recommendations and responses

Surgical Oncology Subcommittee recommendations	DCT response
Increased support for surgical oncology research	RFA "New Approaches in Surgical Oncology" ($1.6 million) issued in 1978
	RFAs "Surgical Oncology Research" ($500,000) for planning grants issued in 1980 and 1983
	Program announcements for surgical oncology research grants (R01, P01) issued in 1981 with re-issue every 4 mos
	Targeted Clinical Investigator Development Award (K08), 1983
	Increased surgical activity in cooperative groups
Initiation of Surgical Evaluation Program in DCT	Surgical Oncology Section established in the Cancer Therapy Evaluation Program, 1978, with $1.6 million portfolio
Establishment of separate Surgical Oncology Study Section	Increased surgical representation on P01 Clinical Section established within Experimental Study Section (1983)
	Establishment of surgical oncology as a cancer activity

funds to foster research in this area. Fifty-one grant applications were reviewed, with 27 applications approved. Priority scores ranged from 208 to 417. The Board of Scientific Counselors recommended payment of grants down to a priority score of 282, recognizing the need to encourage research in surgical oncology. This decision allowed the funding of 11 R01 grants; a total of $955,000 of funds set aside for the RFA was expended. In addition, a $2 million supplement was awarded to cooperative groups to support additional surgical input in cooperative studies.

At the urging of the NCI and DCT, changes were made in the grant review process. Surgeons were added to the primary review committees for clinical R01 and P01 grants in increasing numbers. However, the number of new surgical grants during fiscal year (FY) 1979–1980 did not warrant a separate study section for either type of grant.

The Surgical Oncology Committee did recognize the need to encourage the development of this subspecialty and, as a result of its urging, the Division sponsored a symposium on graduate education in this area. The report of this symposium, as well as a survey of 123 university departments of surgery by the Surgical Oncology Society, were summarized for the Board by Dr. Walter Lawrence at the March 1980 Board meeting. Dr. Lawrence reported that the number of surgical oncologists was inadequate at the national level both in medical centers and community practice, and he stressed the need for training programs to develop leaders in this field.

Dr. Lawrence recommended the establishment of a formal Surgical Oncology Research Development Subcommittee (SORDS) to be composed of both Board members and physicians selected from the surgical community. SORDS was formed at that March 1980 meeting with Dr. Lawrence as chairman. The purpose of SORDS was to assist DCT in long-range planning of surgical oncology research as well as the submission of specific recommendations to the Board to be considered in the process of budget planning by the Director of the Division. The 1983 SORDS membership is shown in table 2.

From its inception, the primary concern of SORDS was the stimulation of support for surgical oncology research, rather than undergraduate education or postgraduate training. SORDS recommended the issuance of a second RFA to stimulate planning of institutional research programs in this field. In October 1980, the concept was approved by the Board of Scientific Counselors of DCT, resulting in an RFA entitled "Surgical Oncology Research" for planning grants. Five hundred thousand dollars was set aside for specific funding of surgical oncology research. Twenty-eight applications were reviewed and 14 were approved, with priority scores ranging from 173 to 346. In 1981, five grants were funded through a priority score of 204 at $481,000. A second RFA for surgical oncology planning was issued this year, again for $500,000.

At the same time, to stimulate interest in research projects (R01s and P01s), a program announcement was also issued in June 1981 calling for grant applications in Surgical Oncology Research. Fifty-one R01 applications were received and nine were funded at $1,181,000, while three P01 applications were reviewed and one was funded at $916,000. As had been recommended by SORDS, these applications were reviewed by special ad hoc committees with significant surgical oncology representation. Traditional R01 and P01 grants in surgical oncology have also been funded through the general grant pool, with total costs of all surgery grants totaling $6.7 million in FY 1983. The June 1981 program announcement was re-issued in late 1983, and will be re-issued at 4-month intervals in the future.

While RFAs and program announcements provide a stimulus for submission of grants, they do not by themselves improve the quality of grants submitted or their competitive position relative to grants in other fields. Money, by itself, does not create good ideas or competitive investigators. It was the common opinion of DCT and SORDS that surgical oncology could flourish as a research endeavor only if talented young surgeons could be attracted to this field in greater numbers and could be given the research background required to compete effectively for grant funding. Therefore, we have taken steps to encourage the development of surgical oncologists as competitive researchers through targeted research training grants.

TABLE 2.—SORDS membership (1983)

Member	Institution
Samuel Wells (chairman)*	Washington University, St. Louis, MO
Ernest deMoss	NCI, Bethesda, MD
Gregory Curt	
Steven Rosenberg	
Dani Bolognesi*	Duke University, Durham, NC
William Shingleton	
Jerome DeCosse	Memorial-Sloan Kettering Cancer Center, New York, NY
William Donegan	Medical College of Wisconsin, Madison
John Durant	Fox Chase Cancer Center, Philadelphia, PA
E. Carmack Holmes	University of California School of Medicine, Los Angeles
Walter Lawrence	Medical College of Virginia, Richmond
Eugene Myers	University of Pittsburgh, PA
Rodrique Mortel*	Hershey Medical Center, Hershey, PA
Barry D. Kahan	M. D. Anderson Hospital and Tumor Institute, Houston, TX
Richard E. Wilson	Brigham and Women's Hospital, Boston, MA

* Member, DCT Board of Scientific Counselors.

Current Efforts and Future Plans in Surgical Oncology Research

The National Research Service Awards support research training in all the sciences relevant to cancer. The research training grant supports both pre- and post-doctoral trainees for a project period of up to 5 years, with the grantee institution choosing the trainees. This mechanism supported 19 training positions in surgical oncology with $430,000 in FY 1983. However, this is only a small fraction (< 10%) of the National Research Service Awards made by NCI. We clearly needed a new type of award, paying a higher salary, and targeted for physicians in postgraduate training.

This year, in cooperation with the Division of Cancer Prevention and Control, we initiated the Clinical Investigator Development Award. This award is designed to encourage recently trained physicians to undertake research careers in oncology, with the goal of developing independent investigatory skills. These awards were specifically designed to develop research abilities in physicians such as surgical oncologists, whose lengthy clinical training has precluded research exposure.

The award includes a $25,000 grant for salary which rises to $30,000 in Years 2-5, with an additional $10,000 per year support for research expenses. The grantee must spend 75% of his or her time in basic research. This funding mechanism will provide the atmosphere in which a surgical oncologist can learn sophisticated laboratory skills from an investigator with experience and expertise in basic science. It is expected that the Clinical Investigator Development Award will be a popular grant mechanism. The first review for the Clinical Investigator Development Award was completed on December 8 and six proposals were considered from physicians in surgical oncology. To assure that a significant number of surgical oncology grants are funded, funds from the Division of Cancer Prevention and Control will be supplemented by DCT to support up to eight meritorious applications from surgical oncologists per year. We believe this award will increase the numbers of academic surgical oncologists who are able to compete successfully for traditional research funding.

Furthermore, both DCT and SORDS felt that it would be important to attract medical undergraduates to the field of surgical oncology through more effective teaching in medical school. At the same time, we hoped to provide support for academic faculty in this field. To accomplish these ends, the NCI added surgical oncology to the list of targeted areas for Clinical Education grants in 1983. Other targeted areas include education in cancer prevention and nutrition. Clinical Education grants totaling up to $6 million per year are available for award to medical schools for faculty salary support and course development in undergraduate education. It is felt that this will prove to be a particularly useful mechanism for emphasizing surgical oncology as a clinical subspecialty and capturing the interest and imagination of medical students when their long-range career plans are still in a formative stage.

The foregoing initiatives have been directed at the problem of encouraging basic research by surgical oncologists, but a second important component, clinical research trials, must also receive increased attention. We have done this primarily through the cooperative and intergroup studies of DCT. Recent efforts have included expansion of activities of cooperative groups with formation of new groups and new membership. Within the groups, program staff are encouraging surgical input. Surgeons serve as group chairmen and principal investigators in five of 21 cooperative groups, including the National Surgical Adjuvant Breast Program, Gynecologic Oncology Group, Lung Cancer Study Group, Prostate Group, and Bladder Group.

Surgical oncologists are also involved in intergroup studies in brain metastasis, soft tissue sarcoma, melanoma, testicular cancer, rectal carcinoma, Wilms' tumor, and rhabdomyosarcoma. The estimated total costs obligated to these surgical groups will be > $11 million in FY 1984. Overall, > 20% of the clinical cooperative trial group budget is provided for surgical studies.

Both the original DCT Subcommittee on Surgery and SORDS have expressed concern that research applications from surgeons might not receive a sympathetic review from NIH study sections, and have asked, at various times, that either a separate study section be established for surgical R01s or that surgical representation be increased on the major committees which review surgical oncology grants. The number of surgical R01s each year is not sufficient to warrant a separate study section. The 50 or so grants received in this area simply would not occupy a committee full time. However, we have seen considerable improvement in the surgical representation on existing committees.

The Experimental Therapeutics Study Section, which sees most of the R01 proposals in the area of cancer treatment, has been divided this year into clinical and preclinical subsections. The section reviewing clinical applications has four surgeons within its 12-person membership.

The other important committees which review clinical grants are the Program Project Review Committee, which has three surgeons among its 20 members (this is consistent with the representation of the other subspecialties and exceeds the proportion of surgical grants reviewed), and the Cancer Clinical Investigation Review Committee, which has four surgeons among its 23 members. The latter committee reviews our cooperative group applications.

Further, in response to the urging of DCT and SORDS, the NCI Executive Committee approved the designation of surgical oncology as a cancer activity during the past year. The establishment of an activity identifies the

TABLE 3.—NCI support of surgical oncology (dollars in thousands)

	Actual	Estimate
Intramural	$ 5531	$ 6252
Extramural		
Research project grants	6738	7150
Cooperative clinical research	8049	8531
Organ systems	86	89
Conference grants	10	10
Cancer control	217	225
Subtotal	$15,100	$16,005
Training		
National Research Service Awards (institutional awards)*	430	590
Clinical Investigator Awards (K08)†	—	162
Subtotal, extramural	$15,530	$16,757
Total, NCI	$21,061	$23,009

*No. of trainees: actual, 19; estimate, 19.

†No. of awards: actual, 0; estimate, 3. Program initiated in FY 1983; no awards were made.

funding of grants in this area as a separate budget item and creates a referral center for surgical oncology grants. The program director, our section head for surgical oncology, can then advise grantees, identify grants which merit funding as exceptions, and initiate special RFAs or program announcements in the activity.

Finally, in an effort to continue dialogue between NCI and the surgical community, SORDS will have an increased role in DCT planning. In addition, the Director of DCT will serve as a member of the Cancer Commission of the American College of Surgeons.

A summary of NCI intramural and extramural support of surgical oncology is shown in table 3. Actual 1983 expenditures of > $21 million are expected to rise to > $23 million in 1984. By continuing to support both basic and clinical research by cancer surgeons, these initiatives will continue to have a positive and measurable effect on the development of surgical oncology.

REFERENCES

1. KILMAN JW, CLATWORTHY HW, JR, NEWTON WA, ET AL. Reasonable surgery for rhabdomyosarcoma: a study of 67 cases. Ann Surg 3:346, 1973.
2. HEYN RM, HOLLAND R, NEWTON WA, ET AL. The role of combined chemotherapy in the treatment of rhabdomyosarcoma in children. Cancer 34:2128–2142, 1974.

Acquisition of Multiple Drug Resistance by CCRF-CEM Cells Selected for Different Degrees of Resistance to Vincristine[1,2]

Valentino Conter and William T. Beck[3,4,*]

We studied the acquisition of multiple drug resistance to several "natural product" drugs by cultured human leukemic lymphoblasts selected for increasing resistance to vincristine (VCR). Three apparent types of cross-resistance patterns could be distinguished: specific, pleiotropic, and mixed. Cross-resistance to vindesine developed in parallel with VCR resistance, appearing at the lowest levels of VCR resistance (approximately fivefold). Vinblastine resistance did not become noticeable until VCR resistance was higher (≈ 50–100-fold). Cross-resistance to three other tubulin-binding agents developed in a different pattern, however. While cross-resistance to maytansine was seen in cells of intermediate (≈ 50-fold) resistance to VCR, colchicine cross-resistance occurred only in cells that were highly resistant to VCR (≈ 500-fold). Even at the highest level of VCR resistance (≈ 600-fold), complete sensitivity to podophyllotoxin was retained. Conversely, cross-resistance to the epipodophyllotoxins, teniposide and etoposide, semisynthetic derivatives of podophyllotoxin, was seen in cells that were > 50-fold resistant to VCR. The same pattern obtained for the anthracyclines, doxorubicin and daunorubicin. We conclude that resistance to low concentrations of VCR does not uniformly confer cross-resistance to other classes of natural product drugs. [Cancer Treat Rep 68:831–839, 1984]

Multiple drug resistance has been described for several experimental tumor systems both in vivo (1–4) and in vitro (5–10). It is usually seen in cloned, homogeneous populations of tumor cells that are often manyfold more resistant to the drug than are the sensitive cells [eg, ≈ 100- to > 100,000-fold (8)]. Whether multiple drug resistance occurs in tumor cells of lower resistance remains unclear. It is important to resolve this question, however, because it is unlikely that drug resistance is expressed to such high degrees in patient tumors, which are presumably heterogeneous mixtures of drug-resistant and drug-sensitive cells (11,12).

Accordingly, we have developed a cell culture model to examine the acquisition of multiple drug resistance in human leukemic lymphoblasts selected by growth in sublethal concentrations of drug for relatively low (approximately fivefold) to relatively high (≈ 600-fold) vincristine (VCR) resistance. We believe that such studies can provide information not only on the development of cross-resistance but also on the ability to detect it, especially at low levels. Our results, some of which have been presented in preliminary form (13), indicate that multiple drug resistance is not acquired in parallel with VCR resistance; ie, cross-resistance to other classes of drugs does not occur until resistance to the selection agent (VCR) is quite high.

MATERIALS AND METHODS

Cells and Culture Conditions

Starting with CCRF-CEM cells, VCR-resistant sublines (CEM/VCR) were selected by growth initially in the continuous presence of sublethal concentrations of VCR, as has been described previously for the selection of vinblastine (VLB)-resistant sublines (14). After 1–2 months,

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when the sublines reached the desired levels of resistance, cells were exposed to drug on an intermittent basis, usually only 3 days/week. All cells were grown in stationary suspension cultures in plastic flasks at 37°C in a humidified chamber gassed with 95% air and 5% CO_2; the minimal essential medium was supplemented with 10% fetal bovine serum. Stock cell cultures were diluted twice weekly, and VCR, at the appropriate concentration, was added to the cultures once weekly. Cells were grown in the absence of drug for 7-10 days before use in an experiment.

Cell numbers were determined with a Coulter Counter (Model ZBI), and thresholds were set to discriminate cells from debris with the aid of a Coulter Channelyzer (Model P1200). The drug sensitivity of the cells was determined as follows. Cells were diluted to a final concentration of 2×10^5/ml in multiwell plates (Costar 3524) and exposed to increasing concentrations of drugs; unless otherwise indicated, cell numbers were determined after 48 hours.

The IC_{50} was calculated as the drug concentration that inhibited the growth of the cells by 50% with respect to the controls. The degree of resistance of the sublines to any drug was calculated by dividing the IC_{50} of the resistant subline by the IC_{50} of the sensitive CEM line. We have found the cross-resistance data to be readily interpretable when the IC_{50} values for a drug are graphed on the ordinate against the different cell lines on the abscissa, ranging from the sensitive CEM cells on the left to the increasingly VCR-resistant on the right.

Chemicals and Supplies

Minimal essential medium, with Earle's spinner salts, was purchased from either Grand Island Biological Co (Grand Island, NY) or KC Biological, Inc (Lexena, KS); fetal bovine serum was purchased from Flow Laboratories (McLean, VA). VLB, VCR, and vindesine (VDS) were generously supplied by Eli Lilly and Co (Indianapolis, IN) through the courtesy of Dr. Gerald L. Thompson. Colchicine (CLC) and podophyllotoxin (POD) were purchased from Sigma Chemical Co (St. Louis, MO), and maytansine (MAYT) was obtained from the National Cancer Institute. Doxorubicin (DOX) and daunorubicin (DNR) were obtained from Adria Laboratories (Wilmington, DE) and cytarabine was obtained from the Upjohn Co (Kalamazoo, MI). These compounds were dissolved in 0.9% NaCl solution prior to their dilution to the final concentration. Teniposide and etoposide were obtained from Bristol Laboratories (Syracuse, NY) through the courtesy of Dr. William T. Bradner, and DMSO was purchased from Fisher Scientific Co (Fair Lawn, NJ). Teniposide and etoposide were first dissolved in 35% DMSO and then diluted in 0.9% NaCl; the cell samples used as a control for the toxicity of these drugs were cultured in the presence of the corresponding concentration of DMSO, which at the highest concentration of 0.69% inhibited cell growth by < 5% of untreated control values. Plastic culture flasks, multiwell plates, and all other supplies were obtained from commercial sources.

RESULTS

Sensitivity of CEM Cells to Drugs

The ranges of sensitivity of the CEM parent line to several classes of "natural" products or their semisynthetic derivatives were established. Cells were exposed to four or five different concentrations of the various drugs for 48 hours, and the IC_{50} values for growth inhibition were determined as described in the Materials and Methods section. The sensitivity of the CEM cells ranged widely, not only between the different classes of drugs (eg, vinca alkaloids and epipodophyllotoxins), but also among drugs of the same class (eg, vinca alkaloids) (table 1). Among

TABLE 1.—Cytotoxicity of various oncolytic agents toward CCRF-CEM cells*

Drug	Molecular weight	IC_{50}† ($M \times 10^{-9}$)	No. of experiments
VCR	923	0.429 ± 0.267	8
VDS	853	0.516 ± 0.217	7
VLB	912	7.07 ± 1.4	4
MAYT	691	0.0056 ± 0.00285	3
CLC	399	14.6 ± 3.7	6
POD	414	25.0 ± 1.0	3
Teniposide	657	111.0 ± 55.0	4
Etoposide	588	204.0 ± 35.0	4
DNR	528	25.9 ± 0.63	7
DOX	556	37.2 ± 0.76	8
Cytarabine	243	18.7 ± 0.23	3

*Cells were grown in multiwell plates for 48 hrs in the absence or presence of several concentrations of the drugs indicated. *See* Materials and Methods section for details.

†The IC_{50} value is the concentration of drug that inhibits the 48-hr cell growth by 50%. This number is derived from a plot of the ratio of drug-treated to control cells on the ordinate vs the drug concentration on the abscissa. Values = mean ± SD.

the vinca alkaloids, which are thought to exert their cytotoxic actions by binding to tubulin (15,16), VLB was about 15-fold less toxic than its analogs, while VDS and VCR were equally effective in inhibiting CEM cell growth. The cytotoxic effects of other tubulin-binding, antimitotic agents also ranged widely. Specifically, MAYT, which is thought to bind to the VCR site on tubulin (17,18), was two orders of magnitude more toxic to the CEM cells than was either VCR or VDS, and 4 $\log_{10}$ units more toxic than either CLC or POD, which both bind to tubulin at sites different from those of the vinca alkaloids (15,19,20). POD, however, was fourfold to eightfold more toxic than its semisynthetic derivatives, teniposide and etoposide, which do not bind to tubulin (21). Finally, the anthracyclines, DOX and DNR, which appear to act not only by binding to DNA (22,23) but also by affecting cell membranes (24–26) or tubulin (27), were as toxic on a molar basis as was cytarabine, which interferes with DNA synthesis, most likely by inhibiting DNA polymerase (28,29).

Growth of VCR-Sensitive and VCR-Resistant Cells

Growth rates of the drug-sensitive CEM parent line, as well as those of several VCR-resistant sublines, were about the same, each having a doubling time of ≈ 24 hours (fig 1). While VCR was progressively toxic to the CEM cells, it had no effect on the growth of the three resistant sublines, CEM/VCR_1, CEM/VCR_{10}, and CEM/VCR_{100}, when present at the selection concentrations of 1, 10, and 100 nM, respectively. (The subscripts refer to the nanomolar selection concentrations.) When grown in the absence of drug for 6 months, however, the CEM/VCR_1, CEM/VCR_{10}, and CEM/VCR_{100} sublines lost 60%, 75%, and 20% of their full resistance, respectively.

Resistance and Cross-Resistance of VCR-Resistant Sublines to Antimicrotubular Agents

While the CEM cells were very sensitive to both VCR and VDS, the VCR-resistant sublines had progressively greater IC_{50} values for these drugs, the CEM/VCR_{100} cells being 653-fold and 1017-fold more resistant, respectively, than were the sensitive CEM cells (fig 2). Conversely, the CEM/VCR_{100} cells were only 19 times more resistant to VLB than was the sensitive CEM parent line, mainly because of the lower intrinsic sensitivity of the parent cells to this drug. Indeed, there was only a twofold difference in the IC_{50} values for VLB and VCR in the CEM/VCR_{100} cells.

In experiments to determine the acquisition of cross-resistance to three other antimicrotubular agents, MAYT, CLC, and POD (fig 3), cross-resistance to MAYT, the most toxic agent tested in this study against the CEM cells (table 1), appeared at low levels of VCR resistance (with the CEM/VCR_5 subline), reaching a maximum with the CEM/VCR_{50} subline. The drug was ≈ 700 times less effective against this subline than against the CEM cells. Conversely, cross-resistance to CLC, which is nearly 4 $\log_{10}$ units less toxic than MAYT in CEM cells, was evident only with the CEM/VCR_{100} subline. This line was only ≈ 11 times less sensitive to CLC than was the CEM

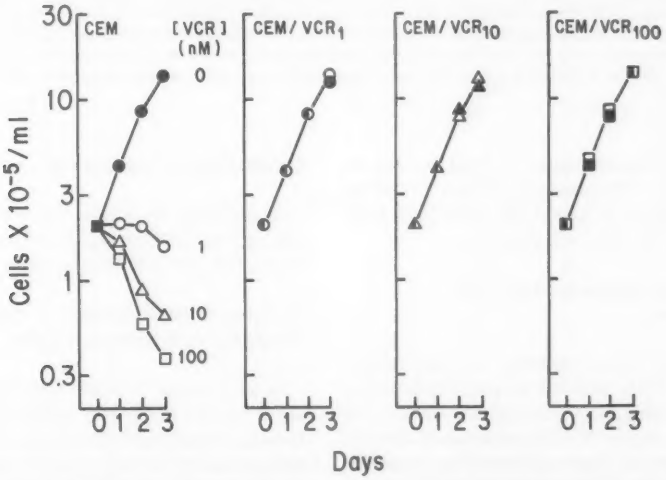

FIGURE 1.—Effects of VCR on the growth of VCR-sensitive (CEM) and VCR-resistant (CEM/VCR) cells. Cells were diluted to 2 × 10^5/ml in multiwell dishes, and drug was added to the final concentration as indicated in the left-hand panel. Cell Nos. were determined daily. Closed symbols represent control cell growth; open symbols represent cell growth in the presence of VCR at the final concentration of: ○ = 1 nM; △ = 10 nM; □ = 100 nM. Subscript numbers of the 3 resistant cell lines refer to the nanomolar selection concentration of VCR.

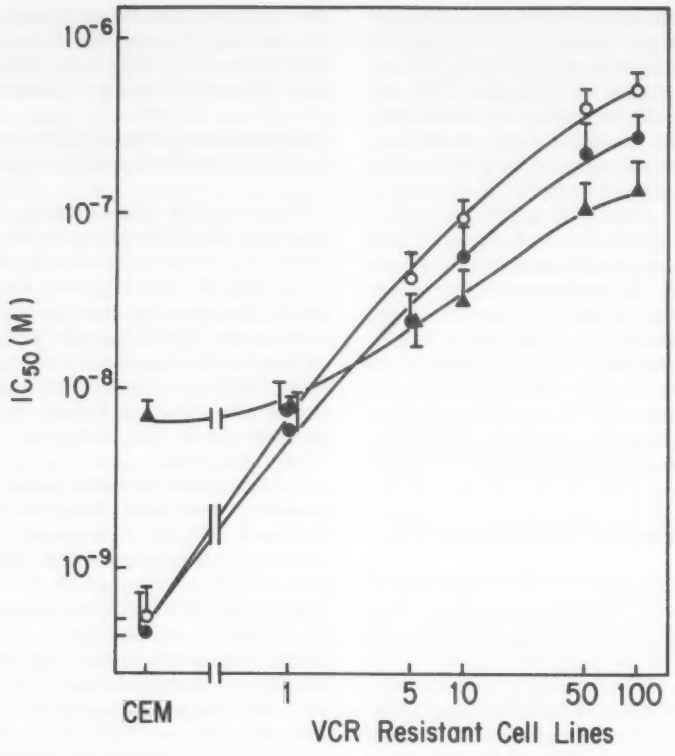

FIGURE 2.—Acquisition of resistance and cross-resistance to vinca alkaloids by VCR-sensitive and VCR-resistant cells. VCR-resistant cell lines 1, 5, 10, 50, and 100, shown on the bottom of the figure, represent CEM/VCR_1, CEM/VCR_5, CEM/VCR_{10}, CEM/VCR_{50}, and CEM/VCR_{100}, respectively. Drug-sensitivity determinations were done as described in Materials and Methods section. The 48-hr IC_{50} was calculated as the drug concentration that inhibited the growth of the cells by 50% with respect to the controls. Symbols: ● = VCR-treated cells; ○ = VDS-treated cells; ▲ = VLB-treated cells. Each point represents the mean of 4–8 separate experiments; bars represent ± 1 SD.

parent line. Finally, cross-resistance to POD was not expressed by any of the VCR-resistant sublines. In this regard, it is of interest that POD was one of the least toxic drugs tested against the CEM cells (table 1).

Cross-Resistance to Anthracyclines and Epipodophyllotoxins

The acquisition of cross-resistance to the anthracyclines, DNR and DOX, and the epipodophyllotoxins, teniposide and etoposide, was qualitatively similar and progressive with the degree of VCR resistance of the subline (fig 4). The degree of cross-resistance also appeared to be related to the intrinsic sensitivity of the parent CEM cells to these compounds. The order of IC_{50} values for these drugs in the sensitive CEM line, DNR < DOX < teniposide < etoposide, held for each VCR-resistant subline tested.

Cytotoxicity of Cytarabine

As expected, no cross-resistance against this nucleoside analog was observed; its IC_{50} value was 1.9×10^{-8} M in both CEM and CEM/VCR_{100} cells.

Patterns of Development of Cross-Resistance to Drugs by VCR-Resistant Cells

In an attempt to relate the acquisition of cross-resistance by the CEM/VCR sublines to the various drugs tested, we compared the degrees of resistance or cross-resistance shown by each resistant subline (fig 5). Two general patterns were evident: cross-resistance to some drugs was acquired at lower levels of VCR resistance, eventually achieving a high degree of cross-resistance (600-fold to 1000-fold for VDS, VCR, and MAYT in the CEM/VCR_{100} cells); cross-resistance to other drugs was

acquired at higher levels of VCR-resistance and was relatively lower (11-fold to 34-fold for CLC, VLB, DOX, DNR, teniposide, and etoposide in the CEM/VCR_{100} cells). As seen in figure 3, cross-resistance to POD did not develop at any level of VCR resistance. When the degree of resistance for each drug in the CEM/VCR_{100} cells was compared with the molecular weights of the drugs, no significant correlation was observed ($r = 0.607, P > 0.05$).

DISCUSSION

We selected cultured human leukemic lymphoblasts for increasing resistance to VCR to determine those levels of VCR resistance at which pleiotropic cross-resistance appears. The selection method was one of initial growth in the continuous presence of sublethal concentrations of VCR for 1-2 months, followed by intermittent treatments with the alkaloid when the desired resistance level was achieved (3 days in drug, 4 days out). We felt that this method might more accurately reflect the clinical acquisition of resistance than standard mutagenesis and cloning protocols and would also offer the advantage of allowing us to have a wide range of VCR resistance to observe the acquisition of multiple drug resistance.

One concern with our method of selection was that the cell population may not have been homogeneous, as possibly reflected in the phenotypic instability of three of the resistant lines, CEM/VCR_1, CEM/VCR_{10}, and CEM/VCR_{100}, noted in the Results section. This appears unlikely, however, because the selection concentrations of VCR (1, 10, and 100 nM, respectively) had no effect on the growth of the resistant sublines (fig 1). More important, the IC_{50} for VCR in the sensitive CEM cells was < 1 nM (table 1), a concentration that is lethal within 3 days (fig 1). Since the resistant cells were in the presence of ≥ 1 nM VCR for several months during the selection process, it is unlikely that any sensitive cells remained. Thus, the phenotypic instability of our resistant lines could be due to double minute chromosomes, which have been shown to be associated with unstable drug resistance (30). Alternatively, in the absence of any VCR selection pressure, resistant cells may have back-mutated to VCR sensitivity (possibly through a loss of double minutes), gaining a growth or survival advantage over

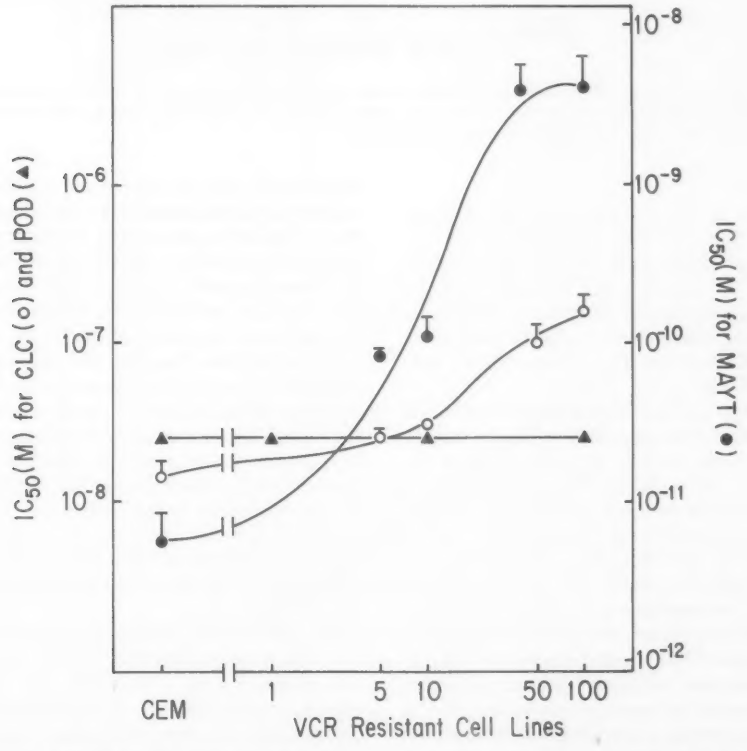

FIGURE 3.—Acquisition of cross-resistance to antimicrotubular agents by VCR-resistant cells. *See* legend to fig 2 and Materials and Methods section for details. Symbols: ● = MAYT-treated cells; ○ = CLC-treated cells; ▲ = POD-treated cells.

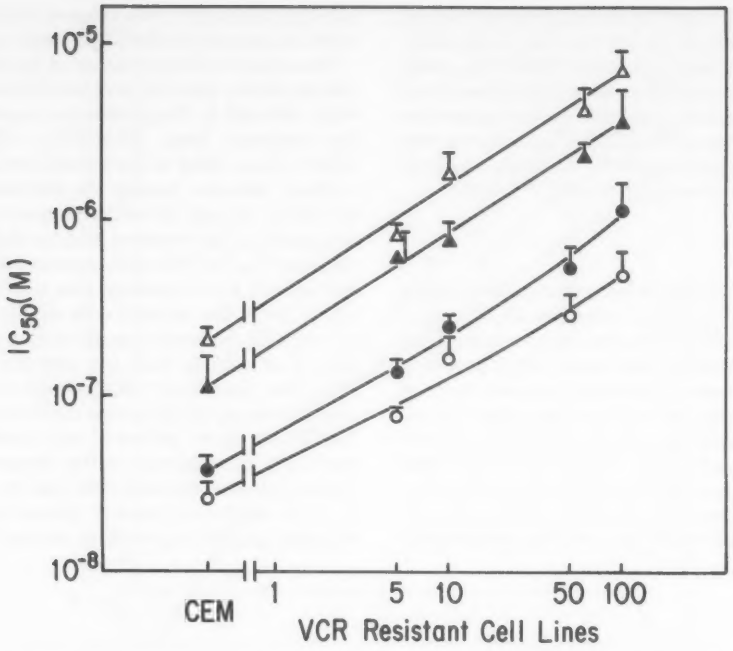

FIGURE 4.—Acquisition of cross-resistance to anthracyclines and epipodophyllotoxins by VCR-resistant cells. *See* legend to fig 2 and Materials and Methods section for details. Symbols: ○ = DNR-treated cells; ● = DOX-treated cells; ▲ = teniposide-treated cells; △ = etoposide-treated cells.

other resistant cells.

Another concern in these studies was that 48-hour growth inhibition is not as valid a measure of cytotoxicity as are cloning experiments (31). Our IC_{50} values, however, at least for etoposide in CEM cells (table 1), agree with those values derived by cytotoxic cloning assays (32), indicating that growth-inhibition assays are adequate for relative comparisons of these cytotoxic compounds in cultured cells.

The VCR-resistant sublines were selected from CEM parent cells that showed a wide spectrum of sensitivity to anticancer drugs, from an IC_{50} of 5.6×10^{-12} M for MAYT to $\approx 1\text{-}2 \times 10^{-7}$ M for the epipodophyllotoxins. Nevertheless, these VCR-resistant sublines were characterized by three patterns of cross-resistance. For VDS, a close structural analog of VCR, against which cross-resistance develops roughly in parallel with VCR resistance, we suggest specific mechanisms such as altered binding to tubulin or some other target, at least at the lower levels of VCR resistance. (VLB, another analog of VCR, appears to be a special case; *see* below.) Such a mechanism has been demonstrated for resistance to demecolcine in CHO cells of low resistance to this drug (33). Conversely, for those drugs to which cells are less cross-resistant at higher levels of VCR resistance (VLB, CLC, DNR, DOX, teniposide, and etoposide), we suggest more general mechanisms, such as altered cell membrane properties, which could cause diminished accumulation of all of these large heterocyclic compounds. In this regard, resistance-associated alterations in cell membrane glycoproteins have been observed in cells selected for resistance to VLB (14), CLC (34), and DOX (35). Finally, MAYT appears to be a compound that bridges both categories. At low levels of VCR-resistance, the drug falls between the "specific" and "general" extremes, whereas at the highest levels of VCR resistance, the drug is clearly associated with VCR and VDS. This interesting cross-resistance behavior may be related to the facts that (*a*) MAYT is not a structural analog of VCR (which may account for the lower degree of cross-resistance in the CEM/VCR_1, CEM/VCR_{-5}, and CEM/VCR_{-10} cells), but (*b*) it is thought to bind to the VCR site on tubulin (17,18). It is not known if any other compounds would display the cross-resistance features of MAYT.

The difference in degree of cross-resistance between the VCR analogs VDS and VLB in the VCR-resistant cells merits comment. VLB falls into the second, more general category of drugs, as seen in figure 5, because the parent CEM cells show a much lower intrinsic sensitivity to VLB than to the other two vinca compounds (table 1). In absolute terms, however, the VCR-resistant cells have similar IC_{50} values for all three vinca alkaloids (fig 2), dif-

fering only by twofold to fourfold in the CEM/VCR$_{100}$ cells. The question remains as to why these parent CEM cells are less sensitive to VLB than to either VCR or VDS. These vinca alkaloids are very much alike in terms of structure and mechanism of action, and both VLB (16) and VDS (36) compete with VCR for binding to tubulin. Although it is well-known that VLB is less effective than VCR against leukemia (37), the reason for this difference at the cellular level has not been defined. However, insights into the bases for these differences have been provided recently by Ferguson et al (38), who showed that VLB was not retained as well as VCR by leukemic cells. These investigators suggested that because VLB is more lipophilic than VCR, more of it may interact with a cellular component (membranes?) unrelated to its presumed primary target, tubulin, and as a consequence, permit the drug's ready dissociation. These observations may help explain the differences between VCR and VLB in our CEM cells (table 1). In accordance with this model, then, progressive alterations in the cell membranes of our VCR-resistant cells, as described previously for VLB resistance (14), may obscure these differences between VCR and VLB by permitting more of each drug to bind to noncytotoxic, secondary cellular targets. Such a model can be tested experimentally.

The results with POD and its derivatives, teniposide and etoposide, also warrant consideration. Regardless of their extent of resistance to VCR, none of the resistant sublines developed cross-resistance to POD. In studies of CHO cells selected for resistance to POD, cross-resistance to the VCR analog, VLB, was demonstrated for some lines but not for others (39,40); resistance appeared to result from an alteration in the cytoplasmic binding of POD, possibly to tubulin. It is possible that in our system any alteration in a VCR binding site on tubulin that may account for VCR resistance will not affect POD toxicity, since POD binds to a site on tubulin different from that of vinca alkaloids (15). Moreover, any putative membrane changes that may contribute to VCR resistance may not have any bearing on the relative cytotoxicity of POD in these cells.

Despite the lack of cross-resistance to POD, cross-resistance to the epipodophyllotoxins was seen, but it reached no more than 30-fold in the highly resistant CEM/VCR$_{100}$ cells (fig 4). In the CEM/VCR$_5$ cells, which were 56-fold resistant to VCR, cross-resistance to teniposide and eto-

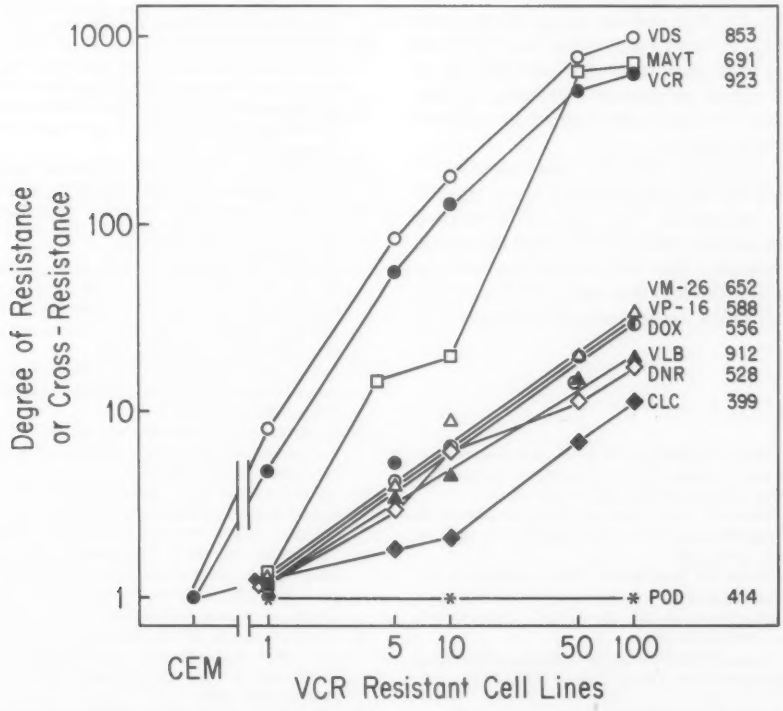

FIGURE 5.—Patterns of cross-resistance to drugs by VCR-resistant cells. Degree of resistance or cross-resistance is expressed as a function of the VCR-resistant subline. *See* text for details. Nos. on the right side of the figure are molecular weights of the drugs.

poside was seen at a lower level, the IC_{50}s always being threefold to fourfold greater than those of the drug-sensitive CEM cells. These results are in general agreement with one study demonstrating that etoposide cross-resistance in VCR-resistant P388 cells was a function of drug concentration and exposure time (41). In contrast, no cross-resistance to either epipodophyllotoxin was observed in P5178Y cells that were 50-fold resistant to VCR (42), suggesting that the cell type may also be important in the development and expression of multiple drug resistance.

Finally, that there was no significant correlation between the molecular weight of a drug and degree of resistance in our CEM/VCR_{100} cell lines (fig 5) contrasts with the earlier findings of Biedler and Riehm (6). This discrepancy may be due to several factors, including differences in cell lines, initial selection agents, and classes of drugs tested. Thus, in the former study, Chinese hamster cells were selected for primary resistance to dactinomycin and were tested against a broad spectrum of drugs, including alkylating agents and a steroid. Based on their data, these investigators suggested that alterations in the cell membrane could account for nonspecific reductions in permeability to cross-resistant drugs (6). Although we found no significant correlation between the molecular weight of a drug and the degree of resistance in our CEM/VCR_{100} cells, we suggest that the acquisition of multiple drug resistance in our cell lines may be related in part to certain progressive membrane or cytoplasmic alterations that affect either the accumulation or the retention of these agents.

In conclusion, the studies reported here appear to enable us to distinguish the acquisition of three types of cross-resistance properties by our VCR-resistant cells: specific, pleiotropic, and "mixed." Most important, multiple-drug resistance does not occur to the same degree as resistance to the selection agent, VCR, and may not be seen at all at the lower levels of VCR resistance examined.

REFERENCES

1. JOHNSON RK, CHITNIS MP, EMBREY WM, ET AL. In vivo characteristics of resistance and cross-resistance of an adriamycin-resistant subline of P388 leukemia. Cancer Treat Rep 62:1535-1547, 1978.
2. KAY SB, and BODEN JA. Cross-resistance between actinomycin D, adriamycin and vincristine in a murine solid tumor in vivo. Biochem Pharmacol 29:1081-1084, 1980.
3. SCHABEL FM, SKIPPER HE, TRADER MW, ET AL. Concepts for controlling drug-resistant tumor cells. *In* Breast Cancer. Experimental and Clinical Aspects (Mouridsen HT, and Palshof T, eds). Oxford, Pergamon Press, 1980, pp 199-211.
4. DANØ K. Cross resistance between vinca alkaloids and anthracyclines in Ehrlich ascites tumor in vivo. Cancer Chemother Rep 56:701-708, 1972.
5. KESSEL D, BOTTERILL V, and WODINSKY I. Uptake and retention of daunomycin by mouse leukemic cells as factors in drug response. Cancer Res 28:938-941, 1968.
6. BIEDLER JL, and RIEHM H. Cellular resistance to actinomycin D in Chinese hamster cells in vitro: cross-resistance, radioautographic, and cytogenetic studies. Cancer Res 30:1174-1184, 1970.
7. LING V, and THOMPSON LH. Reduced permeability in CHO cells as a mechanism of resistance to colchicine. J Cell Physiol 83:103-116, 1974.
8. BIEDLER JL, RIEHM H, PETERSON RHF, ET AL. Membrane-mediated drug resistance and phenotypic reversion to normal growth behavior of Chinese hamster cells. JNCI 55:671-680, 1975.
9. BECH-HANSEN NT, TILL JE, and LING V. Pleiotropic phenotype of colchicine-resistant CHO cells: cross-resistance and collateral sensitivity. J Cell Physiol 88:23-32, 1976.
10. WILKOFF LJ, and DULMADGE EA. Resistance and cross-resistance of cultured leukemia P388 cells to vincristine, adriamycin, adriamycin analogs, and actinomycin D. JNCI 61:1521-1524, 1978.
11. CALABRESI P, DEXTER DL, and HEPPNER GH. Clinical and pharmacological implications of cancer cell differentiation and heterogeneity. Biochem Pharmacol 28:1933-1941, 1979.
12. HART IR, and FIDLER IJ. The implications of tumor heterogeneity for studies on the biology and therapy of cancer metastasis. Biochim Biophys Acta 651:37-50, 1981.
13. CONTER V, and BECK WT. Cross-resistance (CR) properties of CCRF-CEM cells selected for resistance (R) to vincristine (VCR), adriamycin (ADR) and VM-26. Proc Am Assoc Cancer Res 23:197, 1982.
14. BECK WT, MUELLER TJ, and TANZER LR. Altered surface membrane glycoproteins in Vinca alkaloid-resistant human leukemic lymphoblasts. Cancer Res 39:2070-2076, 1979.
15. BRYAN J. Definition of three classes of binding sites in isolated microtubule crystals. Biochemistry 11:2611-2616, 1972.
16. WILSON L, CRESWELL KM, and CHIN D. The mechanism of action of vinblastine. Binding of [acetyl-^{3}H] vinblastine to embryonic chick brain tubulin and tubulin from sea urchin sperm tail outer doublet microtubules. Biochemistry 14:5586-5592, 1975.
17. WOLPERT-DEFILLIPPES MK, BONO VH, DION RL, ET AL. Initial studies on maytansine-induced metaphase arrest in L1210 murine leukemia cells. Biochem Pharmacol 24:1735-1738, 1975.
18. MANDELBAUM-SHAVIT F, WOLPERT-DEFILLIPPES MK, and JOHNS DG. Binding of maytansine to rat brain tubulin. Biochem Biophys Res Commun 72:47-54, 1976.
19. KELLEHER JK. Tubulin binding affinities of podophyllotoxin and colchicine analogues. Mol Pharmacol 13:232-241, 1977.
20. WILSON L. Properties of colchicine binding protein from chick embryo brain. Interactions with vinca alkaloids and podophyllotoxin. Biochemistry 9:4999-5007, 1970.
21. LOIKE JD, and HORWITZ SB. Effects of podophyllotoxin and VP-16-213 on microtubule assembly in vitro and nucleoside transport in HeLa cells. Biochemistry 15:5435-5443, 1976.
22. ZUNINO F. Studies on the mode of interaction of daunomycin with DNA. FEBS Lett 18:249-253, 1971.
23. LIN CC, and VAN DE SANDE JH. Differential fluorescent staining of human chromosomes with daunomycin and adriamycin—the D-bands. Science 190:61-63, 1975.
24. MURPHREE SA, CUNNINGHAM LS, HWANG KM, ET AL. Effects of adriamycin on surface properties of sarcoma 180 ascites cells. Biochem Pharmacol 25:1227-1231, 1976.
25. TOKES ZA, RODGERS KE, and REMBAUM A. Synthesis of adriamycin-coupled polyglutaraldehyde microspheres and evaluation of their cytostatic activity. Proc Natl Acad Sci USA 79:2026-2030, 1982.
26. TRITTON TR, and YEE G. The anticancer agent adriamycin can be actively cytotoxic without entering cells. Science 217:248-250, 1982.
27. NA C, and TIMASHEFF SN. Physical chemical study of daunomycin-tubulin interactions. Arch Biochem Biophys 182:147-154, 1977.
28. FURTH JJ, and COHEN SS. Inhibition of mammalian DNA polymerase by the 5′-triphosphate of 1-β-D-arabinofuranosylcytosine and the 5′-triphosphate of 9-β-D-arabinofuranosyladenine. Cancer Res 28:2061-2067, 1968.
29. GRAHAM FL, and WHITMORE GF. Studies in mouse L-cells on the incorporation of 1-β-D-arabinofuranosylcytosine 5′-triphosphate. Cancer Res 30:2636-2644, 1970.
30. BASKIN F, ROSENBERG RN, and DEV V. Correlation of double minute

chromosomes with unstable multidrug cross-resistance in uptake mutants of neuroblastoma cells. Proc Natl Acad Sci USA 78:3654–3658, 1981.
31. ROPER PR, and DREWINKO B. Comparison of in vitro methods to determine drug-induced cell lethality. Cancer Res 36:2182–2188, 1976.
32. KALWINSKY DK, LOOK AT, DUCORE J, ET AL. Effects of the epipodophyllotoxin VP-16-213 on cell cycle traverse, DNA synthesis, and DNA strand size in cultures of human leukemic lymphoblasts. Cancer Res 43:1592–1597, 1983.
33. KEATES RAB, SARANGI F, and LING V. Structural and functional alterations in microtubule protein from Chinese hamster ovary cell mutants. Proc Natl Acad Sci USA 78:5638–5642, 1981.
34. JULIANO RL, and LING V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochim Biophys Acta 455:152–162, 1976.
35. GARMAN D, and CENTER MS. Alterations in cell surface membranes in Chinese hamster lung cells resistant to adriamycin. Biochem Biophys Res Commun 105:157–163, 1982.
36. OWELLEN RJ, DONIGIAN DW, HARTKE CA, ET AL. Correlation of biologic data with physico-chemical properties among the Vinca alkaloids and their congeners. Biochem Pharmacol 26:1213–1219, 1977.
37. JOHNSON IS, ARMSTRONG JG, GORMAN M, ET AL. The Vinca alkaloids: a new class of oncolytic agents. Cancer Res 23:1390–1427, 1963.
38. FERGUSON P, PHILLIPS R, SELNER M, ET AL. Differential activity of vinca alkaloids against cultured cells. Proc Am Assoc Cancer Res 24:286, 1983.
39. GUPTA RS, HO TKW, MOFFAT MRK, ET AL. Podophyllotoxin resistant mutants of Chinese hamster ovary cells. J Biol Chem 257: 1071–1078, 1982.
40. GUPTA RS. Podophyllotoxin-resistant mutants of Chinese hamster ovary cells: cross-resistance studies with various microtubule inhibitors and podophyllotoxin analogs. Cancer Res 43:505–512, 1983.
41. WILKOFF LJ, and DULMADGE EA. Partial cross-resistance of cultured murine leukemia vincristine-resistant P388 cells to 4′-demethylepipodophyllotoxin ethylidene-β-D-glucoside. Proc Soc Exp Biol Med 164:51–56, 1980.
42. HILL BT, and WHELAN RDH. Establishment of vincristine-resistant and vindesine-resistant lines of murine lymphoblasts in vitro and characterization of their patterns of cross-resistance and drug sensitivities. Cancer Chemother Pharmacol 8:163–169, 1982.

Myocardial Effects of Mitoxantrone and Doxorubicin in the Mouse and Guinea Pig[1]

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Mitoxantrone (DHAQ) was compared to doxorubicin for myocardial effects in the mouse and the guinea pig. Histologically, DHAQ induced a high incidence of focal myocardial damage in mice, similar to that observed with doxorubicin. Functionally, like doxorubicin, DHAQ significantly reduced the rate of contraction and histamine responsiveness of guinea pig right atria in vitro. Additionally, long-term ip administration of either DHAQ or doxorubicin reduced the atrial response to histamine in vitro. These data suggest that DHAQ may have a spectrum of myocardial activity similar to that of doxorubicin. [Cancer Treat Rep 68:841–847, 1984]

Success of the anthracycline antibiotic doxorubicin in the treatment of cancer has prompted the search for oncolytic agents with the wide spectrum of antitumor activity of this drug but without its cardiotoxic effects. A compound from one such search, the aminoanthraquinone derivative mitoxantrone (DHAQ), has been purported to be such a drug. Like doxorubicin, DHAQ was active against a broad range of experimental animal tumors (1). Initial screening data suggested that DHAQ might be slightly less cardiotoxic than doxorubicin, the minimal cumulative cardiotoxic doses in the rat being 18 and ~ 11 mg/kg, respectively (2). It has since been reported by Zbinden and Beilstein (3) that DHAQ, in addition to inducing ECG changes, caused marked alterations in rat heart mitochondria without distortion of myocardial contractile elements or dilatation of the sarcoplasmic reticulum. Additionally, Sparano et al (4) observed ultrastructural myocardial changes in some DHAQ-treated dogs which, unlike observed doxorubicin lesions, did not progress with time and dose. Our experiments were designed to compare DHAQ and doxorubicin for cardiac histological effects in the mouse and for functional effects on guinea pig atrial myocardium.

MATERIALS AND METHODS

Mouse study.—Female Cox ICR mice (Laboratory Animal Supply, Indianapolis, IN) were quarantined for 7 days and weighed 18–22 g at the start of the study. Purina Rodent Chow and water were permitted ad libitum throughout the study. The experimental design was as described by Bertazzoli et al (5). After being randomly divided into five groups, the animals were injected iv with saline (*Group 1*, 0.1 ml/10 g of body weight), doxorubicin (*Group 2*, 2 mg/kg; *Group 3*, 4 mg/kg), or DHAQ (*Group 4*, 2 mg/kg; *Group 5*, 4 mg/kg) on Tuesday and Friday of Weeks 1, 2, 5, 6, and 7; they were killed by ether asphyxiation during Week 11 of the study. Hearts and kidneys were taken at necropsy and fixed in 10% buffered Formalin. Routine paraffin sections (3 μm) of these organs were stained with hematoxylin and eosin and examined microscopically for damage. Myocardial damage was graded as follows: *grade 1*—very slight; scattered, single myocardial fibers with vacuolation or degenerative changes; *grade 2*—slight; scattered small groups of altered myocardial fibers throughout the atrial and ventricular myocardium; *grade 3*—moderate; disseminated myocardial fiber vacuolation or degeneration with only occasional focal unaffected areas; and *grade 4*—marked; confluent groups of affected myocardial fibers; most myocardial fibers affected. Student's nonpaired t test was used to determine the significance of differences in final body weight of control and drug-tested animals. The χ^2 test was used to determine the significance of differences in lesion incidence among treatment groups.

Guinea pig studies.—Male Hartley Strain guinea pigs (Davidson Mill Farm, Jamesburg, NJ) were used in all experiments. The animals were individually caged, with food and water permitted ad libitum. Methods employed

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in isolating and mounting guinea pig right atria and recording atrial contractile rate have been described in detail (6). In brief, the animals were killed by stunning and exsanguination. The atria were mounted vertically in 10-ml isolated organ chambers containing 95% O_2-5% CO_2 aerated McEwan's solution maintained at 33°C-34°C. Grass force displacement transducers and a Beckman R612 Dynograph recorder were used to record atrial contraction.

In vitro.—Guinea pigs used in this study weighed 384-505 g. The right atria were permitted to acclimate for 30-60 minutes before defining the response of each preparation to 3.64×10^{-6} M histamine dihydrochloride (0.1 ml of a 3.64×10^{-4} M solution), a dose previously found to increase atrial contractile rate to approximately 80% of maximum. Atrial rate was determined immediately before and 5 minutes after histamine treatment. The chambers were flushed immediately after the 5-minute reading was taken and every 5 minutes during the 15-minute recovery period. The responsiveness of each preparation to histamine was confirmed and then saline (0.15 ml), doxorubicin, or DHAQ was added to each chamber, the final drug concentration being 1.3×10^{-4} M. Atrial rate was monitored during the 3-hour exposure to drug and then the response of each preparation to 3.64×10^{-6} M histamine in the presence of DHAQ or doxorubicin was determined as described above. Changes in atrial rate were calculated and Student's paired t test was used to define the significance of differences in pre- ($\bar{x}$ of two responses) and post-treatment responses of control and drug-treated atria. The significance of differences in contractile rate of control and drug-treated preparations was determined using Student's nonpaired t test.

Ex vivo.—Details of this method have been reported (6). The animals weighing 268-345 g were randomly divided into three groups: *Group 1*, saline (1 ml/kg); *Group 2*, doxorubicin (1 mg/kg); and *Group 3*, DHAQ (Experiment 1, 1 mg/kg; Experiment 2, 0.5 mg/kg). The animals were injected ip on Days 1, 8, and 15 of the study, observed and weighed daily, and killed 4 days after the last injection. The right atria were isolated, suspended in the chambers, and permitted to acclimate for 30 minutes before their responsiveness to 3.64×10^{-6} M histamine dihydrochloride was defined. Atrial rate was determined immediately before and 5 minutes after histamine treatment, and the change in atrial rate was calculated for each preparation. Student's nonpaired t test was used to determine the significance of differences in basal atrial rate, the chronotropic response of the atria to histamine, and the final body weight of control and drug-treated animals.

Drugs.—DHAQ (free base) was synthesized by Dr. C. C. Cheng, University of Kansas Medical Center, Kansas City. Histamine dihydrochloride was purchased from Sigma Chemical Co, St. Louis, MO. Doxorubicin hydrochloride was obtained from Farmitalia Carlo Erba, Milan, Italy.

RESULTS

Mouse Study

Table 1 summarizes the results of the histological evaluation of heart and kidney sections. No histological evidence of myocardial damage was found in any of the control animals. In contrast, four of 18 (22%) mice treated with a doxorubicin dose of 2 mg/kg × 10 and 13 of 15 (87%) mice treated with a dose of 4 mg/kg × 10 had focal myocardial damage. In the low-dose doxorubicin (2 mg/kg × 10) group, three mice had grade 1 (very slight) and one mouse had grade 2 (slight) myocardial damage. Of the mice treated with 4 mg/kg × 10 of doxorubicin, eight had grade 1, four had grade 2, and one had grade 3 (moderate) myocardial damage, with focal mononuclear cellular infiltration being found in five of the damaged hearts. Myocardial damage was found in 12 of 14 (86%) mice treated with DHAQ (2 mg/kg × 10). Five of these mice had grade 1, four had grade 2, and three had grade 3 myocardial damage. Focal mononuclear cellular infiltration was found in three DHAQ-damaged hearts. Toxicity was observed with 4 mg/kg of iv DHAQ, the first mouse dying 3 days after the second injection. Of the remaining animals in the group, six died 3 days and four died 4 days after the fourth injection of DHAQ.

No treatment-related histological changes were observed in the kidneys of mice given either doxorubicin or DHAQ. In contrast to mice in the 2-mg/kg doxorubicin group (32.2 ± 4.9 g; $\bar{x}$ ± SD), mice in both the 4-mg/kg doxorubicin (27.4 ± 2.3 g) and 2-mg/kg DHAQ (26.1 ± 2.6 g) groups weighed significantly less ($P < 0.001$) than the control (31.6 ± 3.4 g) animals at the end of the 11-week experiment.

Guinea Pig Studies

Results of in vitro experiments to determine the effect of 1.3×10^{-4} M DHAQ or doxorubicin on atrial rate and histamine responsiveness are shown in figure 1. Contractile rate of the control preparations was relatively constant during the 3-hour treatment period, ranging from 188 to 196 beats/minute. Both doxorubicin and DHAQ caused a reduction in basal atrial rate, with significant decreases being observed from 70 and 100 minutes, respectively, after drug administration to the end of the 3-hour treatment period. With doxorubicin, atrial rate decreased with time during the first 115 minutes of exposure to drug, plateauing at approximately 150 beats/minute for the remainder of the treatment period. In contrast to doxorubicin, DHAQ caused a slight transient increase in atrial rate on addition to the chambers. Atrial rate then decreased with time until 130 minutes

TABLE 1.—Histopathological effects of iv doxorubicin and DHAQ on hearts and kidneys of female mice

	Treatment*			
	Saline	Doxorubicin (2 mg/kg)	Doxorubicin (4 mg/kg)	DHAQ (2 mg/kg)
	Heart			
No. of mice examined	10	18	15	14
Incidence of cardiac damage	0%	22%†	87%	86%
No. of hearts with—				
Myocardial vacuolation‡				
Grade 1	0	3	8	5
Grade 2	0	1	4	4
Grade 3	0	0	1	3
Grade 4	0	0	0	0
Focal mononuclear cellular infiltration	0	0	5	3
	Kidney			
No. of mice examined	10	18	15	14
Incidence of renal damage	20%	11%	0%	0%
No. of kidneys with—				
Focal nephritis	1	1	0	0
Focal mineralization, papilla	0	1	0	0
Focal mononuclear cellular infiltration	1	0	0	0

*Dosing schedule of Bertazzoli et al (ref 5); twice weekly (Tuesday and Friday), Weeks 1, 2, 5, 6, and 7. Animals were killed during Week 11 of the study.

†Significantly less than results with 4 mg/kg of doxorubicin, $P < 0.001$ (χ^2 test).

‡*See* Materials and Methods section for description of grades of myocardial vacuolation.

after treatment and remained relatively constant at approximately 158 beats/minute for the remainder of the treatment period. Exposure to either doxorubicin or DHAQ for 3 hours significantly reduced the chronotropic response of the atria to 3.64×10^{-6} M histamine. Relative to pretreatment control responses, the response of the atria to histamine in the presence of 1.3×10^{-4} M doxorubicin or DHAQ was reduced 36% ($P < 0.01$) and 32% ($P < 0.01$), respectively. Unpublished dose-response studies indicate that histamine responsiveness is not related to basal atrial rate. Doxorubicin (4.31×10^{-5} M) significantly decreased atrial rate from 213 ± 15 ($\bar{x} \pm$ SD) to 154 ± 16 beats/minute in 3 hours but did not decrease histamine responsiveness.

Results of an initial experiment comparing doxorubicin and DHAQ at 1 mg/kg ip in the ex vivo guinea pig model are shown in table 2. Toxicity was observed with DHAQ at this dose, with three guinea pigs dying within 3 days after receiving the second injection. The remaining guinea pigs in this group, in addition to an equal number of saline- and doxorubicin-treated animals, were therefore killed on Day 4 after the second injection. Neither DHAQ nor doxorubicin, administered once a week for 2 weeks, had an effect on basal atrial rate. In contrast to doxorubicin, DHAQ caused a significant 33% ($P < 0.05$) decrease in the chronotropic response to 3.64×10^{-6} M histamine. Three weekly ip injections of 1 mg/kg of doxorubicin had no effect on basal atrial rate but caused a 41.3% decrease in histamine responsiveness ($P < 0.01$).

Table 3 summarizes the results of a study comparing 1 mg/kg of doxorubicin to 0.5 mg/kg of DHAQ, injected ip once a week for 3 weeks, for functional effects on guinea pig atrial myocardium. Neither drug had a significant effect on the intrinsic rate of contraction of the right atria. In contrast, both DHAQ and doxorubicin significantly reduced the chronotropic response of the atria to histamine: doxorubicin by 35% ($P < 0.01$) and DHAQ by 25% ($P < 0.05$). The doxorubicin-treated (320 ± 26 g; $\bar{x} \pm$ SD) and DHAQ-treated (372 ± 52 g) animals weighed significantly ($P < 0.001$) less than the control (488 ± 52 g) animals at the end of the study. Three animals from the doxorubicin group died during the experiment, one on Day 11, one on Day 15, and one on Day 16.

DISCUSSION

Our objective was to determine if DHAQ, like doxorubicin, is cardiotoxic. Consequently, DHAQ doses were selected based on our past experience with doxorubicin in the mouse and guinea pig cardiotoxicity models (6) and were intended to be near the maximal tolerated dose, considering the duration of a given experiment. In the mouse, 4 mg/kg iv of DHAQ was toxic, while 2 mg/kg of DHAQ and 4 mg/kg of doxorubicin decreased weight gain but did not cause death during the 11-week experiment. Similarly, 1 mg/kg ip of DHAQ caused death of guinea pigs after the second weekly injection; 0.5 mg/kg of DHAQ, which, like 1 mg/kg of doxorubicin, decreased weight gain during the 3-week experiment, did not cause death.

Three assays were employed in assessing the cardiotoxic potential of DHAQ. The mouse model was used to

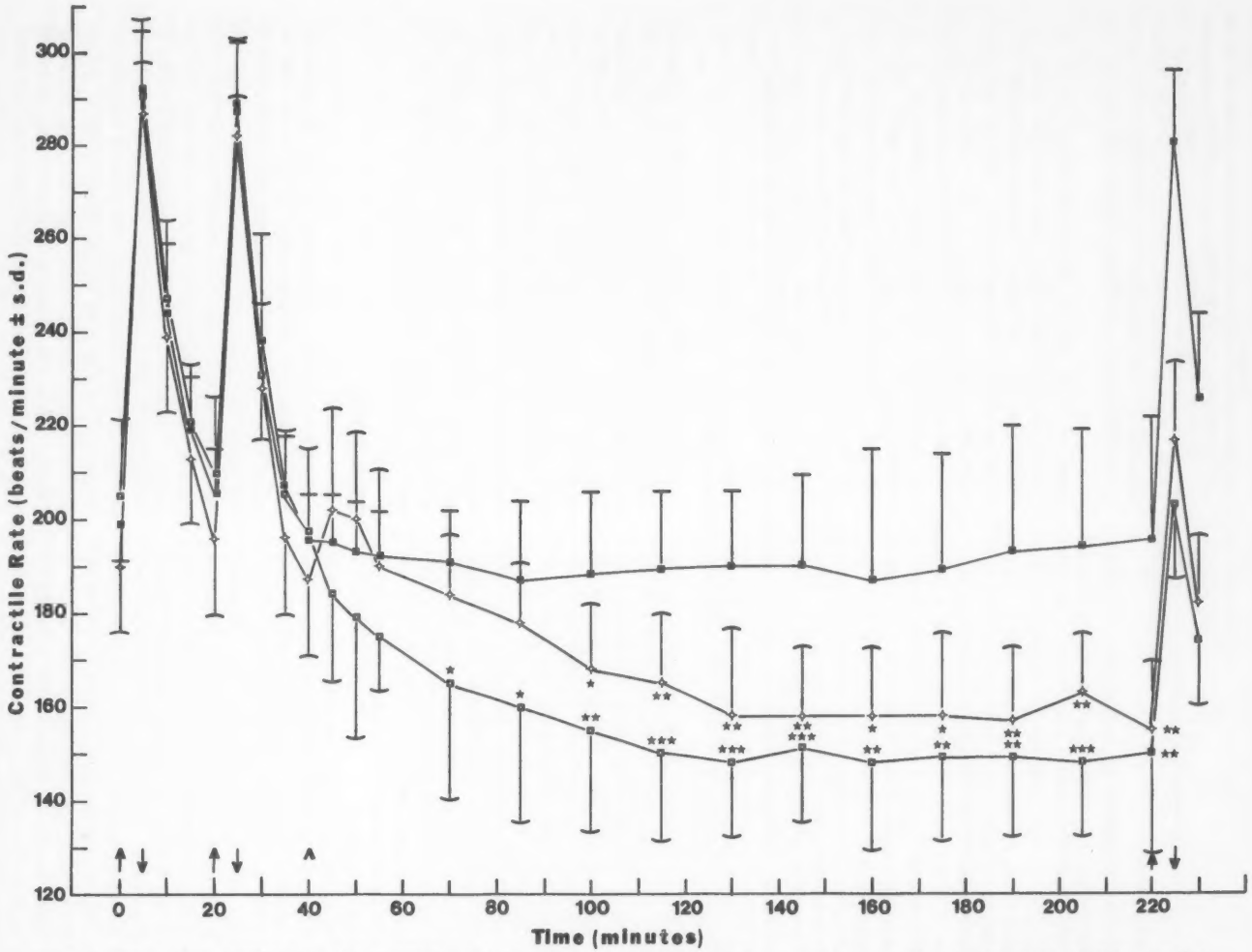

FIGURE 1.—Effect of 1.3×10^{-4} M doxorubicin or DHAQ on isolated guinea pig right atrial contractile rate and response to histamine dihydrochloride (3.64×10^{-6} M). Arrows indicate addition (↑) or removal (↓) of histamine from the chambers. Doxorubicin, DHAQ, or saline (0.15 ml) was added to the chambers at (Δ) 40 mins after starting an experiment. Posttreatment response to histamine was determined in the presence of drug or carrier 3 hrs after addition to chambers. 1 star, 2 stars, or 3 stars indicate that values are significantly different from control: $P < 0.05$, 0.01, and 0.001, respectively. Bath temperatures, 33°C-34°C. ■——■ = saline (n = 8); □——□ = doxorubicin (n = 8); and ○——○ = DHAQ (n = 8).

TABLE 2.—Contractile frequency and histamine responsiveness of isolated right atria from guinea pigs treated ip with doxorubicin (1 mg/kg) or DHAQ (1 mg/kg)

Treatment*	Dose (mg/kg ip)	Contractile frequency (beats/min)				Δ frequency (beats/min) to histamine‡			
		Week 2 (n = 4)†		Week 3 (n = 3)		Week 2 (n = 4)		Week 3 (n = 3)	
		$\bar{x}$ ± SD	% Δ	$\bar{x}$ ± SD	% Δ	$\bar{x}$ ± SC	% Δ	$\bar{x}$ ± SD	% Δ
Saline	—	210 ± 21	—	202 ± 4	—	90 ± 14		92 ±7	
Doxorubicin	1	203 ± 12	-3.6	184 ± 14	-8.9	71 ± 13	-22	54 ± 6	-41.3§
DHAQ	1	197 ± 24	-6.4	—	—	60 ± 12	-33¶	—	—

*Animals injected weekly, for a total of 2 or 3 injections.
†Animals killed on Day 4 following 2nd or 3rd injections.
‡Bath concentration of histamine dihydrochloride, 3.64×10^{-6} M.
§Significantly less than control, $P < 0.01$.
¶Significantly less than control, $P < 0.05$.

TABLE 3.—Contractile frequency and histamine responsiveness of isolated right atria from guinea pigs treated ip with doxorubicin (1 mg/kg) or DHAQ (0.5 mg/kg)

Treatment*	Dose (mg/kg ip)	n	Contractile frequency (beats/min)		Δ frequency (beats/min) to histamine†	
			$\bar{x}$ ± SD	% Δ	$\bar{x}$ ± SD	% Δ
Saline	—	6	201 ± 20	—	92 ± 18	—
Doxorubicin	1	6	190 ± 25	-5.5	60 ± 17	-35‡
DHAQ	0.5	6	195 ± 20	-3.0	69 ± 13	-25§

*Animals treated weekly for 3 wks and killed on Day 4 after 3rd injection.
†Bath concentration of histamine dihydrochloride, 3.64×10^{-6} M.
‡Significantly less than control, $P < 0.01$.
§Significantly less than control, $P < 0.05$.

determine if DHAQ would induce histological damage, and if so, if it was similar to that induced by doxorubicin. The in vitro atrial assay was employed to rapidly determine if DHAQ would induce functional myocardial effects similar to those of doxorubicin. High drug doses were employed to ensure detection of deleterious effects within the 3-hour duration of the assay. The ex vivo guinea pig model was employed to determine if parenteral administration of this drug, like doxorubicin would alter atrial function. It is not known if the effects observed in vitro with high doses of drug correlate with effects observed following long-term drug administration.

Results of our experiments do not support rat (2,3) and dog (4) studies suggesting that the cardiac effect of DHAQ is different from that observed with doxorubicin. Histologically, both DHAQ and doxorubicin caused a similar high incidence of focal myocardial damage (ie, vacuolation and fiber degeneration) in mice. Functionally, both antitumor compounds reduced guinea pig right atrial responsiveness to histamine, and in vitro, basal rate of contraction.

One approach to assessing the cardiotoxic potential of anthracycline-like antitumor drugs is to determine myocardial morphological damage following long-term iv administration of drug. Bertazzoli et al (5) have established a mouse model for this purpose, reporting that cumulative iv doses of 20 and 40 mg/kg of doxorubicin caused a 100% incidence of focal myocardial damage. In our study, these doses of doxorubicin caused cardiac damage in 22% and 87% of the mice, respectively. Myocardial damage identical to that observed with doxorubicin, namely fiber vacuolation and degeneration, was observed in 86% of the animals that received a cumulative dose of 20 mg/kg of DHAQ. Considering that the myocardial damage observed with DHAQ and doxorubicin in this model is essentially similar to that caused by doxorubicin in man (7), it is probable that DHAQ, at some yet undefined cumulative dose, will induce a similar myocardial lesion in man.

A second way of assessing the potential of doxorubicin-like antitumor drugs to cause cardiac damage is to determine their effect on myocardial function. Bossa et al (8) have reported that doxorubicin will decrease the basal rate of contraction of isolated, spontaneously active guinea pig atria. Our in vitro results confirm this observation and show DHAQ to have a similar effect on atrial rate, with the exception that it caused a transient increase in rate on addition to the chambers. Both DHAQ and doxorubicin significantly reduced the chronotropic response of isolated right atria to histamine, both in vitro and following long-term ip administration. These data support our histological observation indicating that DHAQ is similar to doxorubicin with respect to myocardial effects.

Doxorubicin alters cardiac function, and this effect can be detected by stimulating the heart. Papish et al (9) unmasked cardiac abnormalities in doxorubicin-treated patients by increasing systolic blood pressure with iv methoxamine. Breed et al (10) observed reduced maximal apico-basal shortening when isolated, perfused hearts from doxorubicin-treated rats or rabbits were stimulated electrically. Histamine, which is released by doxorubicin administration (11), was employed as an agonist in our studies, as initial in vitro and ex vivo (6) guinea pig experiments indicated that doxorubicin would depress the chronotropic response of the right atria to this drug. Initial unpublished experiments indicate that doxorubicin has a similar depressant effect on the response of the atria to isoproterenol. These observations suggest that the effect of this chemotherapeutic agent is at a site beyond any specific receptor system and that the method employed to stimulate the heart is, therefore, not critical.

No criteria exist at present for selecting a model for assessing the cardiotoxic potential of a compound like DHAQ which is structurally different from, but which has a spectrum of experimental antitumor activity and mechanism of action (ie, DNA intercalation) similar to, doxorubicin (1). Sparano et al (4) evaluated DHAQ for cardiotoxicity in the dog and concluded that it was not cardiotoxic, although myocardial changes (ie, slight dilatation of the sarcoplasmic reticulum) were observed in some dogs treated with DHAQ. Recent clinical reports suggest that DHAQ may be cardiotoxic in man (12–14). Our results suggest that DHAQ is cardiotoxic in the mouse and guinea pig. Thus, it appears that the guinea pig and mouse models may be more predictive than the dog model for assessing the cardiotoxic potential of certain cytotoxic agents.

In summary, our results suggest that DHAQ is similar to doxorubicin with respect to cardiotoxic activity in the mouse and guinea pig. The possibility that the cardiotoxic activities of DHAQ and doxorubicin are synergistic, as suggested by recent clinical reports (12,13), remains to be determined.

REFERENCES

1. WALLACE RE, MURDOCK KC, ANGIER RB, ET AL. Activity of a novel anthracenedione, 1,4-dihydroxy-5,8-bis[[[2[(2-hydroxyethyl)amino]ethyl]amino]]-9,10-anthracenedione dihydrochloride, against experimental tumors in mice. Cancer Res 39:1570–1574, 1979.
2. CHENG CC, ZBINDEN G, and ZEE-CHENG RKY. Comparison of antineoplastic activity of aminoethylaminoanthraquinones and anthracycline antibiotics. J Pharm Sci 68:393–396, 1979.
3. ZBINDEN G, and BEILSTEIN AK. Comparison of cardiotoxicity of two anthracenediones and doxorubicin in rats. Toxicol Lett 11:289–297, 1982.
4. SPARANO BM, GORDON G, HALL C, ET AL. Safety assessment of a new anticancer compound, mitoxantrone, in beagle dogs: comparison with doxorubicin. II. Histologic and ultrastructural pathology. Cancer Treat Rep 66:1145–1158, 1982.
5. BERTAZZOLI C, BELLINI O, MAGRINI U, ET AL. Quantitative experimental evaluation of adriamycin cardiotoxicity in the mouse. Cancer Treat Rep 63:1877–1883, 1979.

6. PERKINS WE, SCHROEDER RL, CARRANO RA, ET AL. Effect of ICRF-187 on doxorubicin-induced myocardial effects in the mouse and guinea pig. Br J Cancer 46:662-667, 1982.
7. FERRANS VJ. Overview of cardiac pathology in relation to anthracycline cardiotoxicity. Cancer Treat Rep. 62:955-961, 1978.
8. BOSSA R, GALATULAS E, and MANTOVANI E. Cardiotoxicity of daunomycin and adriamycin. Neoplasma 24:405-409, 1977.
9. PAPISH SW, BOROW KM, WYNNE J, ET AL. Detection of pre-clinical left ventricular (LV) dysfunction with methoxamine-induced stress in patients (PTS) treated with adriamycin (ADR). Proc Am Assoc Cancer Res and ASCO 22:176, 1981.
10. BREED JGS, ZIMMERMAN ANE, MEYLER FL, ET AL. The interval-force relationship: a technique for evaluating the cardiac toxicity of anthracycline analogs. Cancer Treat Rep 63:869-873, 1979.
11. BRISTOW MR, SAGEMAN WS, SCOTT RH, ET AL. Acute and chronic cardiovascular effects of doxorubicin in the dog: the cardiovascular pharmacology of drug-induced histamine release. J Cardiovasc Pharmacol 2:487-515, 1980.
12. SCHELL FC, YAP H-Y, BLUMENSCHEIN G, ET AL. Potential cardiotoxicity with mitoxantrone. Cancer Treat Rep 66:1641-1643, 1982.
13. PRATT CB, CROM DB, WALLENBERG J, ET AL. Fatal congestive heart failure following mitoxantrone treatment in two children previously treated with doxorubicin and cisplatin. Cancer Treat Rep 67:85-88, 1983.
14. UNVERFERTH DV, UNVERFERTH BJ, BALCERZAK SP, ET AL. Cardiac evaluation of mitoxantrone. Cancer Treat Rep 67:343-350, 1983.

Mechanisms for the Renal Secretion of Cisplatin[1,2]

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Cisplatin (cisDDP) inhibits the active uptake of tetraethylammonium and *p*-aminohippurate by mouse kidney slices. The shapes of the dose-response curves for inhibition of the organic cation and organic anion transport are different, and the inhibition is competitive in each case. Thus, the inhibition by cisDDP may be due to interaction of cisDDP or its aquated products with the specific transport sites rather than to nonspecific metabolic poisoning of the slices by cisDDP. In mice, cimetidine inhibits the renal secretion of tetraethylammonium but fails to inhibit the secretion of *p*-amminohippurate. The renal clearance of total platinum in mice treated with cisDDP did not exceed that of inulin; however, the clearance of platinum was reduced by cimetidine. These results suggest that the organic cation secretory system plays a role in the renal handling of cisDDP or its products. [Cancer Treat Rep 68:849–853, 1984]

Cisplatin (cisDDP) is a useful antitumor agent in humans. The kidney is a site for dose-limiting toxicity in the clinical use of this agent, and a number of methods have been devised to protect that organ so that higher doses, perhaps with greater antitumor effect, may be employed (1). Recently, human (2,3) and rat (4,5) kidneys have been shown to actively secrete cisDDP. If secretion into the renal tubules permits accumulation of higher levels of the drug in kidney tissue, then agents which block secretion may selectively prevent renal toxicity without impairment of the antitumor effect. Therefore, the purpose of this study was to determine the mechanisms by which cisDDP is secreted by the mammalian kidney. Classically, two active, carrier-mediated systems for the renal secretion of organic compounds (often such chemicals are drugs) have been described. These are the organic cation and organic anion systems (6). Classical substrates for the organic cation carrier include tetraethylammonium (TEA) and N^1-methylnicotinamide; classical substrates for the organic anion carrier include *p*-aminohippurate (PAH) and penicillin G. The results obtained suggest that cisDDP, in aqueous solution, is a substrate for both carriers present in mouse kidney slices. Furthermore, the renal excretion of platinum in mice can be inhibited by cimetidine, an inhibitor of the organic cation system. A preliminary report of this work has been presented (7).

METHODS

The uptake of [^{14}C]TEA or [^{14}C]PAH was determined in mouse kidney slices as previously described (8). Male AKR mice from The Jackson Laboratories (Bar Harbor, ME) or outbred ICR mice from TIMCO (Houston, TX) were used. The slices of whole kidneys were preincubated for 15 mins in Ringer's acetate solution at 25°C under an atmosphere of 100% oxygen. The radiolabeled substrate was then added, and the incubation was continued for an additional 30 mins. The slices were removed, blotted on filter paper, and weighed. The slices were then solubilized in 1 ml of NCS tissue solubilizer (Amersham Corp, Arlington Heights, IL) by incubation at 50°C overnight. Radioactivity was determined after adding 19 ml of a toluene-based fluor (Spectrafluor, Amersham Corp). Extracellular space was simultaneously measured in the slices using [^{3}H]inulin, and all values were corrected accordingly.

Renal clearance of free platinum from the plasma of male mice was measured as previously described (9). Essentially, this technique involves the in situ collection of urine in the bladder during a defined time interval. The procedure was designed to determine renal excretion during an interval in which the plasma concentration of free platinum was relatively constant, ie, 60–90 mins after an

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sc dose of 5 mg/kg. Blood was collected from the orbital sinus in groups of eight animals at 60 or 90 mins after cisDDP. Protein-free plasma was prepared by centrifugal ultrafiltration using Centriflo CF-25 Cones (Amicon Corp, Lexington, MA). Total platinum in the protein-free ultrafiltrate and urine was determined by high-performance liquid chromatography after derivatization with diethyldithiocarbamate as described by Bannister et al (10).

cisDDP (Platinol for injection, Bristol Laboratories, Syracuse, NY) was obtained from the M. D. Anderson Hospital Pharmacy. The drug was reconstituted in the incubation medium and used within 4 hours. [^{14}C]TEA (2.86 mCi/mmol) and [^{14}C]PAH (40 mCi/mmol) were purchased from New England Nuclear Corp, Boston, MA. [^{3}H]inulin (3 Ci/mmol) was obtained from Amersham Corp. Cimetidine was a gift from Smith, Kline and French Laboratories, Philadelphia, PA.

RESULTS AND DISCUSSION

cisDDP, at concentrations > 0.1 mM, inhibits TEA uptake by mouse kidney slices (fig 1). The effect of cisDDP on PAH uptake is not observed until a dose > 1 mM is reached, and the dose-response relationship for inhibition of PAH uptake is extremely steep: that is, 1 mM cisDDP is essentially without effect on PAH uptake, whereas 2 mM produces a maximal response. This is in contrast to the dose-response relationship for inhibition of TEA uptake, ie, the effect is expressed over an approximate 1-log dose interval. The different shapes of these dose-effect curves suggest that different mechanisms are operative for the inhibition of TEA or PAH uptake. The inhibition of PAH and TEA uptake produced by another antitumor agent, methotrexate (MTX), is also shown in figure 1. MTX is considered to be a substrate for the organic anion carrier, consistent with the preferential inhibition of PAH uptake shown in figure 1. The whole kidney slice technique used here expresses the organic cation and organic anion systems: that is, PAH, phenol red, and probenecid preferentially inhibit PAH uptake compared to that of TEA. On the other hand, TEA, choline, and N^1-methylnicotinamide inhibit TEA uptake but fail to inhibit PAH uptake. These and other features of the mouse kidney slice system have been presented elsewhere (8).

Since cisDDP is toxic for many types of tissues and cells, it appeared feasible that inhibition of TEA or PAH uptake may occur via a nonspecific metabolic or cytotoxic effect of the drug (11,12). The data given in tables 1 and 2, however, indicate that the inhibition is competitive: that is, TEA protects against cisDDP inhibition of TEA uptake (table 1) and PAH protects against cisDDP inhibition of PAH uptake (table 2). This suggests that the inhibition may be due to specific interaction of cisDDP (or its degradation products) with the organic cation and anion carriers. It was not possible to overcome the cisDDP inhibition of PAH uptake using either PAH or TEA when the cisDDP dose was 2 mM (data not shown).

Previous investigators have attempted to modify the renal secretion of cisDDP using probenecid (13), an in-

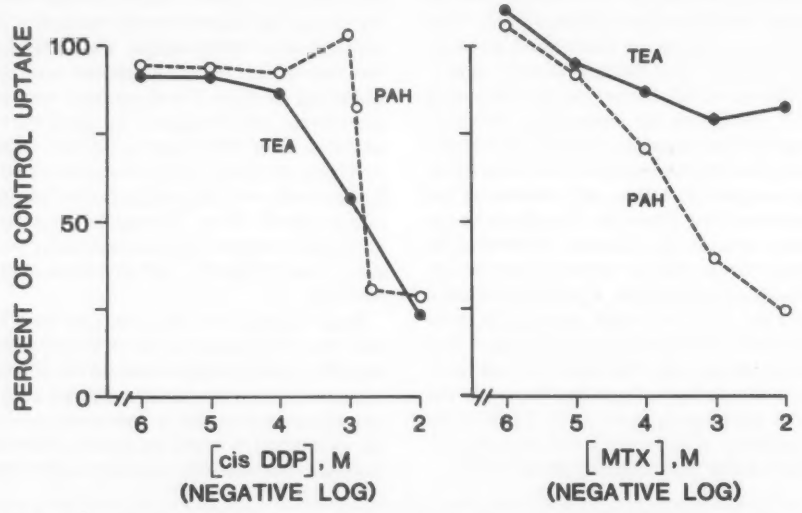

FIGURE 1.—Inhibition by cisDDP of TEA or PAH uptake by mouse kidney slices. [^{14}C]TEA or [^{14}C]PAH (10 μM) were incubated for 30 mins in the presence or absence of various concentrations of cisDDP or MTX as shown. Inhibitors were preincubated with the slices for 15 mins prior to the addition of TEA or PAH as described in the Methods section. Results shown are mean values for at least 4 separate determinations for which the SE was < 10% of the mean value. Tissue to medium concentration ratios were > 5 in controls for both PAH and TEA.

TABLE 1.—Competitive inhibition by cisDDP of the uptake of [^{14}C]TEA by mouse kidney slices

[^{14}C]TEA, μM*	Tissue/medium ratio†		
	Control	cisDDP (1 mM)	% of control
1	29.3 ± 2.1	12.1 ± 2.1	41 ± 5
100	6.93 ± 0.38	5.23 ± 0.29	76 ± 6
500	2.63 ± 0.08	2.35 ± 0.04	89 ± 8

* Mouse kidney slices were incubated in the presence of various concentrations of [^{14}C]TEA for 30 mins as described by Kuttesch et al (8).

†Values = mean ± SE for at least 4 separate determinations.

hibitor of the organic anion secretory system (6); diuretics, such as furosemide; aminophylline; and sodium chloride (14-16). To date, efforts to prevent the renal secretion of cisDDP using an inhibitor of the organic cation secretory system have not been reported, although active uptake of cisDDP by rat kidney slices does appear to involve a process which can be inhibited by organic cations (3). Recently, we (17) and others (18) have reported that cimetidine can be used as an in vivo inhibitor of the secretion of the organic cation TEA without effect on the renal secretion of PAH. The rather selective inhibition by cimetidine of TEA uptake by mouse kidney slices is contrasted with the selective inhibition by probenecid of PAH uptake in figure 2. Consequently, we have employed cimetidine to evaluate the possible role of the organic cation carrier in the renal handling of cisDDP. The renal clearance of platinum following cisDDP is reduced in mice pretreated with cimetidine (table 3). The renal clearance of platinum was 0.517 ml/min in control animals, whereas it was reduced significantly to 0.204 ml/min in animals pretreated with cimetidine. Although the combination of cisDDP and cimetidine appeared to reduce inulin clearance somewhat (from 0.626 to 0.493 ml/min) in these experiments, the reduction in platinum clearance was more pronounced than this apparent effect on glomerular filtration rate.

Using isolated rat kidneys, Daley-Yates and McBrien (5) have recently shown that the renal handling of cisDDP involves tubular reabsorption as well as filtration and secretion. Reabsorption appears to occur via an organic anion system, since probenecid increases the clearance of platinum in rat kidneys (5,19). The renal clearances of total platinum in humans and rats and that of a number of cisDDP metabolites in rats exceed that of creatinine or inulin (2-5), an indication in these species that renal secretion plays a major role in the renal handling of several cisDDP products. Although the renal clearance of total platinum did not exceed that of inulin in mice (table 3), the inhibition of platinum excretion by cimetidine (table 3), coupled with the competitive inhibition by cisDDP of TEA uptake by mouse kidney slices (table 1), suggests that renal secretion of cisDDP or its metabolites occurs (at least in part) via the organic cation system. The possibility exists that this secretion may be selectively inhibited, thereby affording selective protection of the kidney from cisDDP toxicity without impairment of its antitumor activity. To date, potent and selective inhibitors of the organic cation secretory system analogous to probenecid have not been developed; therefore, this premise is difficult to experimentally evaluate at this time. Although large doses (> 100 mg/kg) of cimetidine inhibited the renal secretion of TEA acutely (< 30 mins), the effect dissipated by 60 mins in mice (unpublished data). Thus, cimetidine may not interfere significantly with cation secretion under the usual therapeutic applications of the drug.

TABLE 2.—Competitive inhibition by cisDDP of the uptake of [^{14}C]PAH by mouse kidney slices

[^{14}C]PAH, μM*	Tissue/medium ratio†		
	Control	cisDDP (1.4 mM)	% of control
0.1	6.61 ± 0.93	5.00 ± 0.55	77 ± 5
1.0	6.67 ± 0.87	6.71 ± 0.93	100 ± 2

* Mouse kidney slices were incubated in the presence of 0.1 or 1.0 μM [^{14}C]PAH for 30 mins as described by Kuttesch et al (8).

†Values = mean ± SE for 6 separate determinations.

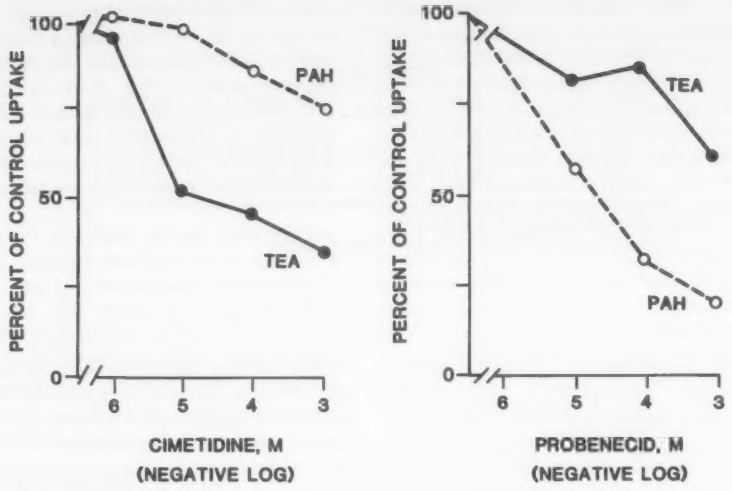

FIGURE 2.—Inhibition of PAH or TEA transport by cimetidine and probenecid. Kidney slices were incubated in Ringer's acetate solution at 25°C in 100% oxygen as previously described (8). Uptake of radioactive PAH or TEA (10 μM) was determined during a 30-min time interval. Results shown are mean values for at least 3 separate determinations for which the SE was < 10% of the mean value.

TABLE 3.—Effect of cimetidine on the renal clearance of cisDDP in mice*

Treatment	Free platinum, plasma† (ng/ml)	Platinum excretion (ng/min)	$C_{platinum}$ (ml/min)	C_{inulin} (ml/min)
Control	211 ± 57	109 ± 24	0.517 ± 0.113	0.626 ± 0.073
Cimetidine	243 ± 73	50 ± 22	0.204 ± 0.092	0.493 ± 0.049

*Renal clearance was determined between 60 and 90 mins after sc administration of cisDDP (5 mg/kg) as described in the Methods section. [^{3}H]inulin (0.5 μCi/mouse, sc) and cimetidine (300 mg/kg, ip) were given 20 mins prior to urethral ligation. Values = mean ± SE. n ≥ 4.

†Mean values ± SE were determined in 4 separate groups of animals (8/group) 60 to 90 mins after cisDDP. Total platinum was measured in protein-free plasma by high-performance liquid chromatography following derivatization with sodium diethyldithiocarbamate (10).

REFERENCES

1. WALKER EM, JR, and GALE GR. Methods of reduction of cisplatin nephrotoxicity. Ann Clin Lab Sci 11:397-410, 1981.
2. JACOBS C, KALMAN SM, TRETTON M, ET AL. Renal handling of *cis*-diamminedichloroplatinum(II). Cancer Treat Rep 64:1223-1226, 1980.
3. JACOBS C, MCGARREY K, RICH L, ET AL. Secretion of cisplatin and effects of probenecid: human and rat kidney slice studies. Proc Am Assoc Cancer Res 23:126, 1982.
4. DALEY-YATES PT, and MCBRIEN DCH. Cisplatin metabolites: a method for their separation and for measurement of their renal clearance in vivo. Biochem Pharmacol 32:181-184, 1983.
5. DALEY-YATES PT, and MCBRIEN DCH. The mechanism of renal clearance of cisplatin (*cis*-dichlorodiammine platinum II) and its modification by furosemide and probenecid. Biochem Pharmacol 31:2243-2246, 1982.
6. MUDGE GH. Drugs affecting renal function and electrolyte metabolism. *In* The Pharmacological Basis of Therapeutics, 6th ed, (Gilman AG, Goodman LS, and Gilman A, eds). New York, MacMillan Publishing Co, 1980, pp 885-892.
7. NELSON JA, SANTOS G, and HERBERT BH. Mechanisms for the renal handling of cisplatin in mice. Proc Am Assoc Cancer Res 24:292, 1983.
8. KUTTESCH JF, JR, ROBINS MJ, and NELSON JA. Renal transport of 2'deoxytubercidin in mice. Biochem Pharmacol 31:3387-3394, 1982.
9. KUTTESCH JF, JR, and NELSON JA. Renal handling of 2'-deoxyadenosine and adenosine in humans and mice. Cancer Chemother Pharmacol 8:221-229, 1982.
10. BANNISTER SJ, STERNSON LA, and REPTA AJ. Urine analysis of platinum species derived from *cis*-dichlorodiammineplatinum (II) by high-performance liquid chromatography following derivatization with sodium diethyldithiocarbamate. J Chromatogr 173:333-342, 1979.
11. GOLDSTEIN RS, NOORDEWIER B, BOND JT, ET AL. *cis*-Dichlorodiammineplatinum nephrotoxicity: time course and dose response of renal functional impairment. Toxicol Appl Pharmacol 60:163-175, 1981.
12. GOLDSTEIN RS, and MAYOR GH. The nephrotoxicity of cisplatin. Life Sci 32:685-690, 1983.

13. ROSS DA, and GALE GR. Reduction of the renal toxicity of *cis*-dichlorodiammineplatinum(II) by probenecid. Cancer Treat Rep 63:781-787, 1979.
14. PERA MF, ZOOK BC, and HARDER HC. Effects of mannitol or furosemide diuresis on the nephrotoxicity and physiological disposition of *cis*-dichlorodiammineplatinum (II) in rats. Cancer Res 39:1269-1278, 1979.
15. HEIDEMANN H, GERKENS J, and BRANCH R. Prevention of *cis*-platinum nephrotoxicity by aminophylline, furosemide and high salt diet—possible role of tubuloglomerular feedback. Clin Res 30:253A, 1982.
16. EARHART RH, MARTIN PA, TUTSCH KD, ET AL. Improvement in the therapeutic index of cisplatin by pharmacologically induced chloruresis in the rat. Cancer Res 43:1187-1194, 1983.
17. NELSON JA, KUTTESCH JF, JR, and HERBERT BH. Renal secretion of purine nucleosides in mice. Biochem Pharmacol 32:2323-2327, 1983.
18. WEINER IM, and ROTH L. Renal excretion of cimetidine. J Pharmacol Exp Ther 216:516-520, 1980.
19. OSMAN NM, and LITTERST CL. Effect of probenecid and N^1-methylnicotinamide on renal handling of *cis*-dichlorodiammineplatinum-II in rats. Cancer Lett 19:107-111, 1983.

Use of an In Vitro Technique To Detect Mutations Induced by Antineoplastic Drugs in Mouse Germ Cells[1,2]

L. S. Goldstein[3,4,*]

Male mice were given graded doses of procarbazine or mechlorethamine in the presence or absence of prednisolone. Eight to 24 days later, these mice were mated with untreated females and the resulting zygotes were cultured in vitro from the two-cell stage to an early implantation stage. The frequency of successful embryonic development in these embryos was less than the frequency in embryos sired by untreated control animals. The reduced frequency reflects the induction of mutagenic lesions in the germinal epithelium by the drugs and their transmission by the spermatozoa to the oocyte. [Cancer Treat Rep 68:855–858, 1984]

Many drugs used in cancer therapy have known genetic effects in mammalian germ cells (1). Although few data are available, there is little reason to believe that the drugs would be less mutagenic in humans than they are in other animals. Although mutagenesis tests using bacteria, yeast, or cultured cells may be of predictive value, only testing in the whole animal can faithfully represent the distribution of presumptive mutagen and its metabolic products in all germ cell stages.

Many test systems have been designed using rodents but all share common shortcomings: they are expensive and require facilities and expertise that preclude their being undertaken by a small or even moderate-sized laboratory. To overcome these impediments, I have designed an in vitro protocol for a test that detects radiation (2) and alkylating agent-induced (3) mutations in postmeiotic spermatogenic cells in animals. The mutational lesion is detected as a reduced frequency of successful development in vitro of embryos derived from mutated germ cells, relative to the frequency of successful development in vitro of embryos from undamaged germ cells. The mutational lesion detected results from chromosomal damage and is known as a dominant lethal mutation (DLM).

Two common chemotherapeutic agents with mutagenic action in animal experiments are procarbazine and mechlorethamine (4). These are used in combination with vincristine and prednisolone in MOPP therapy for treating Hodgkin's lymphoma. Their mutagenic hazard in a clinical context is still unclear, since procarbazine has been shown to be positively mutagenic only at doses that exceed those administered clinically, and the dose of mechlorethamine found to be mutagenic is just subtoxic in the mouse (4). Since the genetic risks of the therapy are important in evaluating the quality of life, especially in the young patient who has been cured of his disease, I have tested these drugs at clinical dosages in my assay to see if mutagenic effects can be detected by the in vitro method. To mimic MOPP, the effect of prednisolone was also investigated.

METHODS

Male mice, strain Dub:(ICR), 6–8 weeks old, were given graded doses of procarbazine (Matulane, Hoffmann-La Roche, Inc) or mechlorethamine (Mustargen, Merck and Co, Inc) in the presence or absence of prednisolone. Procarbazine (30–150 mg/kg) and prednisolone (8.3 mg/kg) were injected ip; mechlorethamine (0.3–4.0 mg/kg) was injected into the tail vein. The range of dosages was chosen to cover in mouse-equivalents (5) the doses administered in a single course of MOPP (6).

Seven to 20 days after drug treatment, the mice were evaluated for induced DLMs. Fertilizing spermatozoa in matings at these times were derived from treated spermatids (7). The males were paired twice each week with females that had been induced to superovulate (8). Successful matings were determined by the presence of a copulatory plug the following morning. One day later,

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[4]The expert technical assistance of Mrs. Joan Florence and Ms. Joann Charron-Vennari is appreciated.

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the females were killed and their oviducts were excised. Two-cell stage zygotes were flushed from the oviduct by irrigating with modified Hanks' balanced salt solution delivered by a 30-gauge needle inserted into the ampulla. The zygotes were counted, transferred to standard egg culture medium, and cultured for 3 days, during which time they develop to the blastocyst stage. They were then transferred to modified Eagle's medium and incubated for 4 days, during which time they hatch from the zona pellucida and attach to the culture substrate by the trophectoderm, the fetal contribution to the placenta. Many trophectoderm outgrowths also have an inner cell mass, the progenitor of the embryo itself. All incubations were done in a humidified 5% CO_2 and 95% air atmosphere at 37°C. Details of these procedures have been published (2,8).

DLMs expressed in vitro are detected as a reduced frequency of successful development in the group of embryos sired by treated compared to control males (2). On Day 3 of culture, blastocysts, morulae, and dead or arrested zygotes were counted in each group. On Day 7, trophectoderm outgrowths and inner cell masses were counted.

DLM rates were determined by applying the following relationships:

$$DLM_p = 1 - \left[\frac{\left(\frac{\text{sum of blastocysts and morulae/}}{\text{No. of two-cell stage, experimental}}\right)}{\left(\frac{\text{sum of blastocysts and morulae/}}{\text{No. of two-cell stage, control}}\right)}\right]$$

$$DLM_i = 1 - \left[\frac{\left(\frac{\text{No. of trophectoderm outgrowths/}}{\text{No. of blastocysts, experimental}}\right)}{\left(\frac{\text{No. of trophectoderm outgrowths/}}{\text{No. of blastocysts, control}}\right)}\right]$$

$$DLM_e = 1 - \left[\frac{\left(\frac{\text{No. of inner cell masses/}}{\text{No. of trophectoderm outgrowths, experimental}}\right)}{\left(\frac{\text{No. of inner cell masses/}}{\text{No. of trophectoderm outgrowths, control}}\right)}\right]$$

$$DLM_o = 1 - \left[\frac{\left(\frac{\text{No. of inner cell masses/}}{\text{No. of two-cell stage, experimental}}\right)}{\left(\frac{\text{No. of inner cell masses/}}{\text{No. of two-cell stage, control}}\right)}\right]$$

where DLM_p, DLM_i, DLM_e, and DLM_o represent the DLM rate for failures in preimplantation stages, failures at implantation, failures at early postimplantation, or overall failures (between fertilization and early implantation), respectively.

RESULTS

Prednisolone.—The developmental potential of embryos sired by control males injected with prednisolone alone was not different from that of embryos sired by their non-prednisolone-injected counterparts. The developmental potential of embryos sired by drug-treated males that had received prednisolone was not different from that of embryos sired by males that had received the drug alone. Therefore, the prednisolone data and non-prednisolone data for each group were combined.

Procarbazine (fig 1).—The fertilization rate (ie, the ratio of two-cell-stage zygotes to the sum of two-cell stage zygotes and unfertilized ova) of spermatozoa derived from procarbazine-treated spermatids was similar to that of controls. A total of 22,645 two-cell stage zygotes were assayed for DLMs. DLMs expressed before blastocyst formation (DLM_p) or between blastocyst and formation of a trophectoderm outgrowth (DLM_i) were not detected at significant levels even at the highest dose. In contrast, DLMs expressed mostly as a failure to differentiate and proliferate an inner cell mass (DLM_e and DLM_o) were found at significant levels. When considering the mutation-induction rate as a function of dose for DLM_e or DLM_o, a linear increase is seen between 60 and 150 mg/kg after a threshold at doses < 60 mg/kg. This same relationship is suggested for the earlier end points also.

Mechlorethamine (fig 2).—The fertilization rate of spermatozoa derived from mechlorethamine-treated spermatids was the same as that of controls, except for

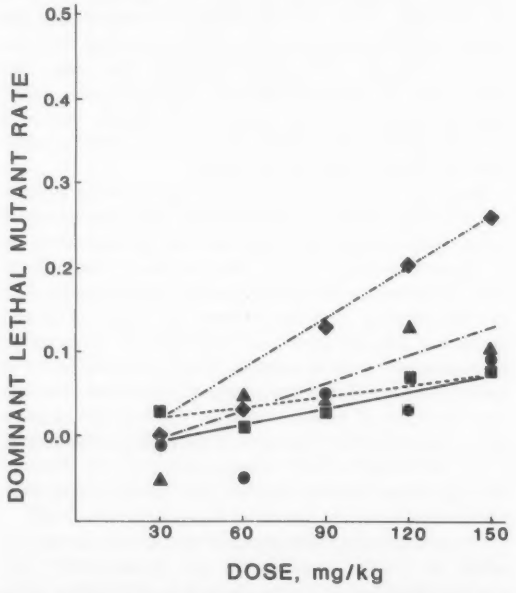

FIGURE 1.—Mutant rates for embryos sired by male mice treated with 30-150 mg/kg of procarbazine. ●——● = mutations expessed between the 2-cell stage and blastocyst formations; ■ ---- ■ = mutations expressed between the blastocyst stage and the formation of a trophectoderm outgrowth; ▲ -·- ▲ = mutations expressed as a failure of an inner cell mass to differentiate and proliferate atop a trophectoderm outgrowth; and ◆ -·-·- ◆ = mutations expressed at any time between the 2-cell stage and differentiation and proliferation of an inner cell mass atop the trophectoderm outgrowth.

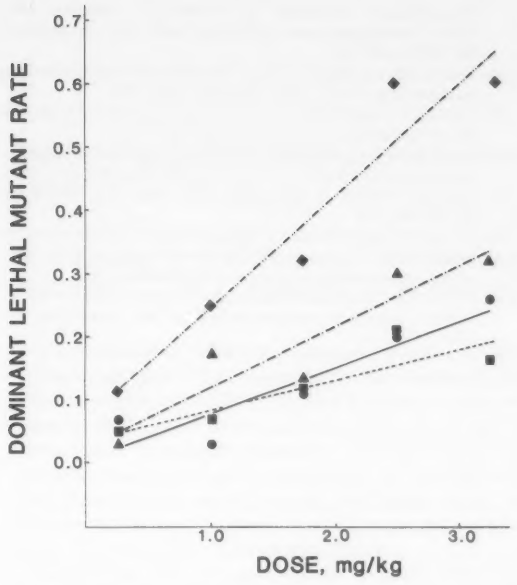

FIGURE 2.—Mutant rate for embryos sired by male mice treated with 0.1–3.75 mg/kg of mechlorethamine. End points are described in legend for fig 1.

the highest drug doses where a moderate reduction was found. A total of 17,115 two-cell-stage zygotes were entered in the assay. Significant rates of mutation induction were found for all end points in embryos fertilized by spermatozoa that were derived from mechlorethamine-treated spermatids. The rate for DLM_p and DLM_i were both about one-half of the rate for DLM_e. The overall rate was about threefold higher than the rates for DLM_p and DLM_i. No evidence for a threshold dose was found.

DISCUSSION

In vivo tests show many agents to be effective inducers of DLMs (4). Significant levels of mutation induction by these drugs are detected by the in vitro methodology, and the drug doses giving positive results are within the range of doses administered in a single course of MOPP therapy (6).

It is not clear whether the manifestation of embryo death is related to a specific drug action. Procarbazine inhibits DNA synthesis (9), an action of limited significance under my experimental conditions since there is no active DNA synthesis in the postmeiotic spermatid. There is a demonstrable mutagenic effect, however, suggesting either that the drug is bound and transmitted by the spermatozoa to the oocyte where it then acts or that some other drug action damages the male pronucleus. In either event this damage is expressed as a failure to differentiate and proliferate an inner cell mass (fig 1). The observation that procarbazine induces specific locus mutations (10) suggests that a mechanism involving base-mispairing may be involved. Mechlorethamine is a potent DNA alkylator and cross-linking agent (11) that does not require active DNA synthesis for its activity. Since less potent alkylating agents have been shown to break chromosomes in spermatids (12), one cannot restrict the activity of mechlorethamine to alkylation alone. Disruption of development in cleavage, which is one aspect of mutation induction by mechlorethamine (fig 2), has been associated with genetic damage at the level of the chromosome (12).

The nature of embryo failure bears on the utility of conventional in vivo dominant lethal tests to describe fully the mutagenic hazard. In vivo tests evaluate only postimplantation losses, since these tests cannot separate a failure due to a DLM that is expressed before implantation from a failure to fertilize the oocyte. Both of these effects are reflected by a decrease in the frequency of implantations relative to corpora lutea, and the inability to discriminate these different effects renders evaluations in the preimplantation stages unreliable (13). Our data (figs 1 and 2) indicate that many of the mutations induced by mechlorethamine would not be detected in in vivo protocols that evaluate postimplantation losses. Since most procarbazine-induced mutations affect only postimplantation development, the in vivo test would be adequate.

Mutations in the spermatid stage do not represent the long-term hazard to the spermatogonial stem cell. Although the spermatid stage is particularly sensitive to DLM induction by several alkylating agents, mutations induced by antineoplastic drugs in this stage may not always be detected in treated spermatogonia (14). While this may reflect the inadequacies of the in vivo tests as discussed before, it may also reflect differential accessibility of the drug or differential repair of potentially mutagenic lesions by the various spermatogenic stages (14). The in vitro methodology can be applied directly to evaluations of mutation induction in stem cell spermatogonia by analyzing the developmental potential of embryos sired ≥ 5 weeks after treatment (7). The long-term genetic hazard of these drugs in spermatogonia is being investigated.

REFERENCES

1. SEIBER SM, and ADAMSON RH. Toxicity of antineoplastic agents in man: chromosomal aberrations, antifertility effects, congenital malformations, and carcinogenic potential. Adv Cancer Res 22:57–155, 1975.
2. GOLDSTEIN LS, and SPINDLE AI. Detection of x-ray induced dominant lethal mutations in mice: an in vitro approach. Mutat Res 41:289–296, 1976.
3. GOLDSTEIN LS. Methyl methanesulfonate-induced dominant lethal mutations in male mice detected in vitro. Mutat Res 42:135–138, 1977.

4. EPSTEIN SS, ARNOLD E, ANDRE J, ET AL. Detection of chemical mutagens by the dominant lethal assay in the mouse. Toxicol Appl Pharmacol 23:288–325, 1972.
5. FREIREICH EJ, GEHAN EA, RALL DP, ET AL. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man. Cancer Chemother Rep 50:219–244, 1966.
6. HUGULEY CH, DURANT JR, MOORES RR, ET AL. A comparison of nitrogen mustard, vincristine, procarbazine, and prednisone (MOPP) vs. nitrogen mustard in advanced Hodgkin's disease. Cancer 36: 1227–1240, 1975.
7. OAKBERG EF. Duration of spermatogenesis in the mouse and timing of stages of the cycle of the seminiferous epithelium. Am J Anat 99:507–516, 1956.
8. GOLDSTEIN LS, SPINDLE AI, and PEDERSEN RA. X-ray sensitivity of the preimplantation mouse embryo in vitro. Radiat Res 62:276–287, 1975.
9. KREIS W. Mechanism of action of procarbazine. *In* Proceedings of the Chemotherapy Conference on Procarbazine (Matulane: NSC-77213): Development and Application (Carter SK, ed). Bethesda, MD, 1970, pp 35–44.
10. EHLING UH, and NEUHAUSER A. Procarbazine-induced specific-locus mutations in male mice. Mutat Res 59:245–256, 1979.
11. LAWLEY PD. Some chemical aspects of dose-response relationships in alkylating mutagenesis. Mutat Res 23:283–295, 1974.
12. BREWEN JG, PAYNE HS, JONES KD, ET AL. Studies on chemically induced dominant lethality. I. The cytogenetic basis of MMS-induced dominant lethality in post-meiotic male germ cells. Mutat Res 33:239–250, 1975.
13. EPSTEIN SS, and ROHRBORN G. Recommended procedures for testing genetic hazards from chemicals, based on the induction of dominant lethal mutations in mammals. Nature 230:459–460, 1971.
14. EHLING UH. Differential spermatogenic response of mice to the induction of mutations by antineoplastic drugs. Mutat Res 26:285–295, 1974.

Multiple Sequential Biomarkers in Monitoring Patients With Carcinoma of the Lung[1,2,3]

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To correlate serial biomarkers and disease activity in carcinoma of the lung, carcinoembryonic antigen (CEA), neuron-specific enolase (NSE), adrenocorticotropic hormone (ACTH), C3-derived protein (C3DP-C), and LDH were assayed in 43 patients with small cell lung carcinoma (SCLC) and in 20 patients with non-small cell lung cancer (NSCLC) (15 with adenocarcinoma, three with squamous cell carcinoma, and two with mixed histology). Disease status after treatment was rated as one of the following: complete response, partial response, minor regression, stable disease, and progressive disease. Significant correlations between disease status and markers in SCLC were found for CEA, NSE, LDH, and ACTH. In NSCLC, only CEA and LDH showed significant correlation. Marker-marker correlations were significant in SCLC for CEA and NSE ($P < 0.05$), CEA and LDH ($P = 0.01$), and NSE and LDH ($P < 0.01$); in NSCLC none were significant. None of the markers exhibited significant correlations with specific metastatic sites. Certain biomarkers (CEA, NSE, and LDH in SCLC; CEA and LDH in NSCLC) can be used alone or in combination to monitor disease activity but appear to be no more sensitive than standard clinical investigational methods. [Cancer Treat Rep 68:859–866, 1984]

An increasing number of tumor products are being recognized for their potential value as biomarkers that are useful in diagnosing and staging of disease, monitoring of therapeutic efficacy both during and after therapy, and possibly predicting early relapse. The now routine clinical use of human chorionic gonadotropin in choriocarcinoma in women and of HCG and alpha-fetoprotein in testicular germ cell tumors in men exemplifies this utility. However, specific, sensitive, and reliable biomarkers are lacking for the vast majority of common cancers, including lung cancers.

Although numerous tumor products have been associated with small cell lung carcinoma (SCLC) and to a lesser degree with non-small cell lung cancer (NSCLC) (1), as yet none has proven to be an adequate biomarker which has clinical utility in diagnosing and monitoring of disease. To improve the utility of biomarkers in the clinical setting, there has been a search for and an evaluation of new markers, a further characterization of known tumor products, and an evaluation of combinations of new and existing markers to determine if multiple marker analyses would improve clinical application over single-marker analysis. In the current study we utilized all of these approaches by the simultaneous analysis of five biomarkers in patients with lung cancer. These assessments were performed blindly and without influenc-

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ing clinical treatment and were correlated with standard clinical staging and investigations so that the clinical usefulness of the biomarkers could be assessed in addition to their biological behavior.

The products chosen for study were carcinoembryonic antigen (CEA), LDH, adrenocorticotropic hormone (ACTH), neuron-specific enolase (NSE), and a DNA-binding form of complement component C3DP-C. Of these, CEA and LDH have been the most studied and have been suggested to have value as markers of disease activity, disease extent, and prognosis in a variety of tumors, including lung tumors (2-11). Levels of ACTH have also been variously shown to reflect changes in the activity of lung carcinomas, although it is subject to non-tumor-related clinical variations (12-14). C3DP-C and NSE are the most recently characterized tumor products in our study. C3DP-C has recently been found to be elevated in a number of cancers and to correlate with the course of disease (15,16). NSE is produced in considerable quantities by all types of "apudomas" but not by nonendocrine tumors (17,18). Thus, on theoretical grounds, NSE could be the most potentially useful marker in SCLC; one recent study has shown it to be of value for monitoring disease activity in SCLC (19).

MATERIALS AND METHODS

From January 1979 through August 1981, 63 sequential unselected consenting patients with bronchogenic carcinoma referred to the University of Maryland Cancer Center (UMCC) for treatment had at least one assay of one of the following biomarkers: CEA, NSE, ACTH, C3DP-C, and LDH. The respective normal values for these markers in our laboratories are: CEA, < 2.5 ng/ml of plasma (< 5.0 ng/ml for smokers); NSE, < 12 ng/ml of serum; ACTH, < 100 pg/ml of plasma; C3DP-C, 5-20 μg/ml of serum; and LDH, 30-120 units/ml of serum. When clinically possible, serial assays were performed at 4-6-week intervals. Concurrently, and at additional times as dictated by clinical events, other routine biochemical, hematological, radiological, scanning test, and clinical reassessments of tumor activity were performed.

Each of the 43 patients with histologically confirmed SCLC received one of four similar and equally efficacious combinations of doxorubicin, cyclophosphamide, and etoposide with or without additional treatments (20). The 20 patients with NSCLC [15 with adenocarcinoma, three with squamous cell carcinoma (SCC), and two with mixed histologies (one with adenocarcinoma + SCC; one with SCLC + SCC which behaved clinically as SCC)] received one or more of the following therapies either alone or in combination: radiotherapy; cisplatin with or without vindesine; doxorubicin; cyclophosphamide; etoposide with or without cisplatin; or hyperthermia with cyclophosphamide and doxorubicin (21,22).

Standard criteria for response were used. Complete remission (CR) was defined as the disappearance of all signs and symptoms of disease lasting ≥ 30 days. Partial response (PR) was defined as a minimum of 50% decrease in the cross-perpendicular dimensions of all lesions with subjective improvement and no evidence of progression, lasting a minimum of 30 days. Minor regression was defined as any unequivocal tumor regression which did not fulfill the criteria of at least PR. Stable disease was defined as the absence of objective regression and of increased tumor growth during therapy, lasting at least 60 days. Progressive disease was defined as an increase of at least 25% in the size of lesions or the appearance of new lesions.

All study patients had measurable or evaluable disease. During the assay periods for patients with SCLC 17 patients had CRs, 13 had PRs, six had minor regressions, and two had stable disease. Five patients with SCLC had assays on only one occasion, two before starting treatment and three during progressive disease. In patients with NSCLC two patients had CRs, one had a PR, six had minor regressions, nine had stable disease, and two had progressive disease. Of these, four patients with NSCLC had assays on only one occasion, one during minor response, one with stable disease, and two during progressive disease.

Biomarker Assays

CEA was assayed by the Hansen Z-gel method with the use of reagents and procedures provided with the CEA-Roche kit (Hoffmann-LaRoche, Inc). Radioimmunoassays were used to measure NSE (23) and ACTH (Amersham Corp). C3DP-C was determined by the method of Parsons et al (16). Total LDH assays were performed using the Calbiochem-Behring LDH-L-SVR system.

All patients were normocalcemic and clinically euvolemic. ACTH assays performed while patients were receiving exogenous corticosteroids were excluded from all analyses.

Statistical Methods

Clinical and laboratory data and patient status assessments from patients at the UMCC were combined with marker data for the same patients performed in a blinded fashion at the Biological Markers Program of the National Cancer Institute-Frederick Cancer Research Facility. The marker data, by identity, represent an accumulation of values from multiple patients at different points in the course of the disease. Thus, different sets of patients contribute to each disease status point and not to cohort serial observations in individual patients. All the analyses were done using the Statistical Analysis System of the Division of Computer Resources and Technology, National Institutes of Health, Bethesda, MD.

Two sets of correlations were computed for measure-

ments paired by time of specimen collection for each patient. These correlations were patient clinical response status versus marker levels and marker versus marker levels. Spearman and Kendall correlations were used in all calculations using remission status, because of the noncontinuous nature of the status assessments (24). Pearson correlations were used for marker versus marker correlations. Within each histology type, a tally of the number of patients with significant correlations (at P = 0.1, 0.05, 0.025, 0.01, 0.005, and 0.001) at each measurement pair was made. After correlations were run for each patient separately, a P value was assigned to the ratio of the number of significant correlations per number of correlations performed (25).

One-way analyses of variance were performed on chemistry measurements and marker levels grouped by response category. The same analyses were also performed to determine the effect of number of metastatic sites on biomarker measurements.

Means and standard deviations of biomarker levels within each response category for each histologic type were computed and t tests were performed for significant differences between pairs of these means. The mean marker levels for patients with metastasis at a given site was tested using a t test against the mean marker level for patients without metastasis at the same site. Metastatic sites tested included brain, liver, nodes, bone, and bone marrow.

RESULTS

The total number of assays performed for each biomarker and the number of patients from which these assays were drawn are shown in table 1. The numbers of patients having three or more assays of a given marker are separately indicated. For each marker, > 70% of patients had three or more assays. Where more than one pretreatment sample was obtained, these typically showed ≤ 10% variation for individuals and a mean was used for the purpose of assigning normality or abnormality.

Tables 2 (SCLC) and 3 (NSCLC) show the means, standard errors, ranges, and numbers of patients for the respective biomarkers, divided into the five disease activity plus pretreatment categories.

For SCLC (table 2), a progressive decrease in the numerical values of means and ranges with decreasing disease activity is apparent for CEA and LDH (using Spearman and Kendall correlations of statistical significance at the 0.05 level). For NSE this decrease in the numerical values applies only for pretreatment versus all other categories. For all other markers there is no definite trend. In addition, ACTH showed a significant positive correlation with progressive disease. In addition, the following marker-marker correlations by Pearson correlation were statistically significant: CEA and NSE ($P < 0.05$), CEA and LDH ($P = 0.01$), and NSE and LDH ($P < 0.01$). Isolated correlations of disease status with ACTH or C3DP-C occurred but did not consistently reflect changes in disease status.

In table 3 (NSCLC), similar trends for CEA and LDH are seen (statistically significant Spearman and Kendall correlation at the 0.05 level). None of the other markers showed positive trends or significant correlations, and even on an individual patient basis none of the biomarkers consistently reflected the changing course of disease. In patients with NSCLC, all marker-marker correlations by Pearson correlation were also negative.

For all patients, multiple combinations and permutations of markers were studied in search of more significant correlations with disease activity than afforded by single markers; however, none of these were superior to single biomarkers. Correlations were found only to be as good as those of the best marker in any combination.

Eighteen patients with SCLC (three with limited disease and 15 with extensive disease) had at least one pretreatment assay that was markedly elevated over control values; the proportion of patients with increased levels is shown in table 4. Of the seven SCLC patients with elevated pretreatment NSE, all except one had elevated CEA and LDH. Pretreatment elevations of C3DP-C in SCLC were usually minor (C3DP-C values: 20, 21, 29, 32, 34, and 37 μg/ml) and all were accompanied by elevated CEA.

TABLE 1.—Biomarker assays results

	CEA	NSE	ACTH	C3DP-C	LDH
SCLC patients					
Total No. of assays	355	176	314	153	278
No. of patients with ≥ 1 assay	41	20	41	22	40
Average No. of assays/patient	8.2	8.8	7.7	7.0	7.0
No. (%) of patients with ≥ 3 assays	34 (83)	17 (85)	31 (76)	16 (73)	29 (73)
NSCLC patients					
Total No. of assays	136	59	130	73	127
No. of patients with ≥ 1 assay	20	7	20	13	20
Average No. of assays/patient	6.8	8.4	6.5	5.6	6.4
No. (%) of patients with ≥ 3 assays	16 (80)	7 (100)	16 (80)	10 (77)	16 (80)
Normal values	50 ng/ml of plasma	12.0 ng/ml of serum	100 pg/ml of plasma	5-20 μg/ml of serum	30-120 units/ml of serum

TABLE 2.—Biomarker results by disease activity category in SCLC

	Pretreatment	Progressive disease	Stable disease	PR	CR	Minor response	Overall
CEA (ng/ml)							
No. of patients	40	66	56	27	82	81	349
Mean	41	35.5	13	7	6	3	16
SE	16	10	3	3	1.5	0.3	2.9
Range	0.7-595	0.5-469	0.5-116	0.2-8.9	0-99	0-24	0-595
NSE (ng/ml)							
No. of patients	14	42	38	9.0	50	42	196
Mean	98	36	24	24	15	19	28
SE	35	12	2.5	6.6	1.7	2.4	4.0
Range	7.2-520	6.0-530	7.0-73	5.0-67	0.8-75	5.0-75	0.8-350
C3DP-C (μg/ml)							
No. of patients	11	20	36	9	27	43	146
Mean	33	29	28.5	25	26	28.5	28
SE	3.8	3.1	2.5	2.1	1.5	2.1	1.1
Range	20-58	7-55	4-73	16	13-45	11-84	4-84
ACTH (pg/ml)							
No. of patients	43	59	59	25	77	79	338
Mean	75	148	60	69	70	146	100
SE	8.3	19	8.3	14	7	63	15
Range	0-215	0-804	0-282	0-264	0-277	0-5000	0-5000
LDH (units/ml)							
No. of patients	31	62	63	24	74	65	317
Mean	233	228	135	139	111	90	152
SE	45	43	17	14	4.5	2.4	13
Range	75-1485	63-2875	65-995	73-345	60-276	55-168	55-2875

Seven patients with SCLC had elevated pretreatment ACTH. All pretreatment ACTH elevations occurred together with increased CEA or LDH or both.

In pretreatment patients with NSCLC, elevations of CEA and LDH also tended to occur together. NSE elevations occurred in two patients (19 and 40 ng/ml) with adenocarcinoma metastatic to bone and brain, prior to all therapy. Both patients received whole-brain irradiation and systemic chemotherapy achieving stable systemic disease and minor response. The former patient had even higher levels following whole-brain irradiation and during chemotherapy (42, 43, 57, and 37 ng/ml), which decreased to 12 ng/ml at progressive disease. The second patient maintained a fairly constant elevation (36, 17, 33, and 38 ng/ml) after whole-brain irradiation and during chemotherapy and also exhibited a fall at progressive disease (9 and 14 ng/ml).

The relatively limited amount of pretreatment data was analyzed for correlations between the number of metastatic sites and marker levels, and although none reached significance, there were trends in patients with SCLC for NSE and LDH and in patients with NSCLC for CEA and LDH. Statistical analyses by metastatic site failed to achieve significance.

Relatively few patients had sequential assays performed throughout the entire course of their disease (pretreatment, maximum remission, and relapse). Table 5 shows the number of patients with sequential analysis and the behavior of their markers during disease regression and subsequent progression. Pretreatment levels were not necessarily increased in these patients. A significant change in biomarker level during regression or progression was defined as a > 50% decrease or increase in level, respectively. In fact, all positive correlations exceeded a 100% change. It is apparent that a fall in marker level with regression was not always associated with a subsequent rise at disease progression and that a failure of levels to fall during remission did not preclude a later rise with progression. When only those patients with pretreatment elevations of CEA or LDH were assessed the associations were also not statistically significant. The small numbers, however, preclude definitive conclusions.

The biomarker data were assessed by correlation of the clinical data for their potential to "predict" or "anticipate" progressive disease. For CEA this occurred in only one patient each with SCLC and NSCLC. In both cases "anticipation" occurred 1 month prior to clinical progressive disease. In some additional patients, suggestions of progressive disease initially appeared but could not be definitely distinguished from random fluctuations seen during clinically stable periods of CR, PR, minor response, or stable disease.

Spurious (unaccountable and isolated) high biomarker levels (2-4 × upper limit of normal) were common for ACTH (15 patients with SCLC and seven with NSCLC) but also occurred for CEA (two patients with SCLC and

TABLE 3.—Biomarker results by disease activity category in NSCLC

	Pretreatment	Progressive disease	Stable disease	Minor response	PR	CR	Overall
CEA (ng/ml)							
No. of patients	15	25	56	11	40	10	125
Mean	365	125	33	137	53	1.1	104
SE	365	42	10	97	1.5	0.25	45
Range	0.8-5353	2.8-973	0-450	2.5-1082	1.6-9.0	0-2.3	0-5353
NSE (ng/ml)							
No. of patients	4	19	27	4	—	5	59
Mean	29	21	21	33	—	21	22.3
SE	4.4	3.9	2.6	3.1	—	9.2	1.9
Range	19-40	5-62	6.3-57	24-38	—	7.5-57	5-62
C3DP-C (μg/ml)							
No. of patients	11	16	31	9	—	5	73
Mean	36	35	30	29	—	31	32
SE	3.7	4.3	1.8	9.2	—	4.7	1.4
Range	22-58	15-72	6-64	14-44	—	23-47	6-72
ACTH (pg/ml)							
No. of patients	18	25	56	11	4	8	125
Mean	63	71	80	79	119	99	78
SE	15	13	8	18	43	36	6
Range	0-215	0-271	0-298	0-169	49-245	22-258	0-298
LDH (units/ml)							
No. of patients	16	31	70	13	5	6	141
Mean	148	152	127	162	120	88	137
SE	28	16	8	37	10	6	7
Range	81-541	62-512	41-394	78-596	96-158	69-108	41-596

one with NSCLC). On only one occasion, however, did such spurious elevations occur in two consecutive assays, and none occurred in more than two consecutive assays. In these circumstances, the other multiple markers were stable and it was thus concluded that these were spurious elevations rather than true covert progressive disease. Furthermore, subsequent clinical data failed to show the predictive value of the spurious results.

For most of the patients in the study, records of smoking habits before and at diagnosis were correlated with biomarker levels. Analysis of variances for the smoking patients with SCLC were divided into 16 groups according to total pack years (0-100 pack years; 1 pack year = 1 pack/day for 1 year). These groups showed statistically significant, positive associations for CEA ($P < 0.0001$), NSE ($P < 0.0001$), C3DP-C ($P < 0.05$), and LDH ($P < 0.01$). For the NSCLC patients, by 10-pack year groups, the respective correlations were: CEA ($P = 0.068$), NSE ($P < 0.001$), and LDH ($P < 0.001$). When SCLC and NSCLC were assessed within respective disease activity categories (CR, PR, minor response, etc), statistically significant correlations were seen only for CEA among SCLC patients with $P < 0.001$ for all disease activity and pretreatment categories. Among the NSCLC patients, only pretreatment and stable disease categories had sufficient data for this analysis, with significant correlations in stable disease for NSE ($P < 0.001$), LDH ($P < 0.01$), and CEA ($P < 0.05$), and in pretreatment for LDH ($P < 0.05$) and CEA ($P < 0.05$).

DISCUSSION

CEA.—Since the demonstration of CEA in 1965 by Gold and Freedman (26), CEA has been extensively evaluated in a variety of cancers of the embryonic gut, including those of the lung. There have been a number of studies showing positive correlations of disease stage or extent with CEA levels (2-7), less commonly with specific metastatic sites (3,7), and rarely with subsequent survival (27). Relatively few studies in lung carcinoma have

TABLE 4.—Pretreatment biomarker assays*

	CEA	NSE	ACTH	C3DP-C	LDH
SCLC	16/17 (94)	7/7 (100)	7/18 (39)	6/6 (100)	12/14 (86)
NSCLC	8/9 (89)	2/2 (100)	4/9 (44)	5/5 (100)	5/8 (63)

* Values = No. elevated/total No. tested (%).

TABLE 5.—Clinically positive correlations of biomarkers during tumor regression, progression, or both

Biomarker	No. of evaluable patients	Nos. of positive correlations		
		Regression*	Progression†	Both‡
SCLC				
CEA	9	7	5	5
NSE	3	1	1	0
ACTH	9	1	2	1
C3DP-C	7	0	0	0
LDH	9	1	5	4
NSCLC				
CEA	5	1	3	1
NSE	2	0	0	0
ACTH	5	1	1	0
C3DP-C	4	0	1	0
LDH	4	1	1	1

*Pretreatment to maximum regression. Note that pretreatment values were not necessarily elevated.

†Maximum regression to progressive disease.

‡Patients exhibiting correlations of markers during both regression and progression.

specifically looked at CEA to monitor and predict the progress of disease. These few studies, however, have generally indicated that CEA levels vary in accordance with changes in disease status and occasionally precede the clinical recognition of changes in disease status by up to 3 months (2–4). Our findings are consistent with these earlier findings in both SCLC and NSCLC but only for the use of CEA as a monitor of disease activity. There was no significant correlation with clinically occult progressive disease. Some of the differences between this study and the foregoing reports might be due in part to the relatively small amount of pretreatment data in the current study, as well as the monthly assessment of CEA compared to the weekly assessment of patients clinically. This disparity between the frequencies of clinical and biochemical assessments would tend to reduce the chances of detecting a change in marker level prior to the clinical recognition of a change in disease status. However, it would also suggest that frequent clinical investigation of patients is at least as sensitive in detecting tumor regression or progression as the more costly and difficult assessment of one or more biomarkers at monthly intervals. These considerations apply equally to all biomarkers studied in this report.

NSE.—NSE is a neuronal form of the glycolytic enzyme enolase, which was first found in extracts of brain tissue and was later shown to be present in APUD (amine precursor uptake and decarboxylation) cells as well as neurons of the neuroendocrine system, but not in other peripheral cells (17,18). Since SCLC is believed to derive from cells of the APUD system, NSE is potentially one of the specific markers for that disease. One clinical study of NSE levels in patients with SCLC supports this possibility (19). In that study, NSE was increased in 69% of 94 untreated patients with SCLC and serial measurements in 23 patients showed an excellent correlation between NSE and clinical response. Our study showed seven of seven pretreatment NSE elevations in patients with SCLC but also two of two in patients with NSCLC, thus raising a question about the specificity of NSE for SCLC. It is possible that the two patients with NSCLC had unrecognized elements of SCLC but this was not apparent even after either clinical or histological review of the pretreatment specimen. However, both these patients had cerebral metastases and were receiving cranial irradiation during the assay period. If intracranial disease may have increased the peripheral NSE levels, it could point to the possible use of NSE in the management of intracranial metastases. Equally, it is possible that cranial irradiation, by its action on either normal or intracerebral tumor tissue, can influence NSE levels. Further study of these questions is warranted. As a potential biomarker in monitoring SCLC, our study indicates that NSE is as good as, but not better than, CEA or LDH. In addition, our results suggest that smoking might raise the levels of NSE as it does for CEA. Thus, NSE is apparently not the ideal biomarker for SCLC, although further study is warranted.

C3DP-C.—Elevated human serum DNA-binding protein has been associated with a variety of malignant diseases including carcinomas, leukemias, lymphomas, and melanomas. Parsons et al (15,16,28) reported an average level of 242 ng/ml (range, 146–400) in patients with active carcinomas, an average of 83 (range, 40–146) in normal human sera, and normal or only slightly increased levels in nonmalignant disease. They also found that 80%–90% of individuals with cancer, including two of two with lung cancers who experienced disease regression, had a decline of C3DP-C to normal values. Moreover, nonresponders retained high C3DP-C levels and

> 80% of patients with progressive disease had a rise in C3DP-C levels, including one of two patients with lung cancers. In our study C3DP-C was elevated in the sera of six of six pretreatment SCLC patients and in the sera of five of five NSCLC patients. No positive correlations with disease activity were found for C3DP-C. The lack of positive findings, however, suggests the need for continued refinement and study of this assay.

ACTH.—Ectopic ACTH and its associated Cushing's syndrome was the first ectopic hormone and clinical paraendocrine syndrome to be intensively studied in lung cancer; ACTH has now been demonstrated in the tumors and plasma of a substantial percentage of patients with lung cancer, although a clinical syndrome of hypersteroid secretion occurs in < 5% of SCLC patients (13,29,30). Several studies have looked at the potential use of ACTH assays to monitor the activity of lung cancers or to assist in the assessment of disease extent, response, and survival. Gropp et al (14) found ACTH assays useful in monitoring antitumor therapy, including the "prediction" of relapse by several weeks, but they found no relation to disease stage. Abeloff et al (13) found four positive correlations with disease progression after PR in five SCLC patients but found no correlation with regression of disease. In contrast, Hansen et al (30) found no correlation of pretreatment ACTH levels with survival, progression, or with response to therapy. In our study, ACTH was increased in about 40% of pretreatment SCLC and NSCLC patients and a significant positive correlation of ACTH level with progression of disease was present in SCLC patients. It would seem unlikely that ACTH assays will have a major role in the assessment and monitoring of lung carcinomas; however, in selected individuals ACTH could be a useful clinical adjunct if steroids are not used.

LDH.—There have been surprisingly few studies to assess the usefulness of LDH in monitoring the progress of patients with lung cancer. Gill et al (31) studied 17 patients with cancer including two with lung cancer and found a good correlation between clinical progress and LDH activity. This included an association of CR with a return to normal LDH levels and isoenzyme distributions. Cohen et al (9) reported a significant correlation between higher LDH levels and a worse prognosis in SCLC. The usefulness of LDH as an indicator of disease has been better established for lymphomas, leukemias, and testicular cancer (10,11,32,33). In our patients, LDH was also a useful monitor of tumor activity in both SCLC and NSCLC and was at least as good as any of the other markers examined for that purpose.

CONCLUSIONS

In SCLC, CEA, NSE, and LDH (and ACTH to a lesser degree) exhibited significant positive associations with tumor activity. In NSCLC the correlations were restricted to CEA and LDH. When compared with the routine clinical examinations and biochemical, radiological, and isotopic scanning assessments performed regularly as part of usual patient care in our clinic, these markers often provided adjunctive data supporting the clinical assessments of disease activity. Taken alone or in combination, however, these markers were no more sensitive than standard clinical methods. At this stage, one must consider that the routine use of these assays outside of research protocols cannot be justified.

Although these markers do show some correlation with disease activity, considerable research remains to be done to find reliable biomarkers that will follow tumor burden to much lower levels (eg, $< 10^6$ cells), predict early relapse, allow for early changes in treatment, and help to establish diagnosis in difficult cases. Several markers which have recently been identified as potential candidates from this and other studies include C3DP-C, NSE, and bombesin (34–36).

REFERENCES

1. SORENSON GD, PETTENGILL OS, BRINCK-JOHNSEN T, ET AL. Hormone production by cultures of small-cell carcinoma of the lung. Cancer 47:1289–1296, 1981.
2. WAALKES TP, ABELOFF MD, WOO KB, ET AL. Carcinoembryonic antigen for monitoring patients with small cell carcinoma of the lung during treatment. Cancer Res 40:4420–4427, 1980.
3. GOSLIN RH, O'BRIEN MJ, SKARIN AT, ET AL. Plasma carcinoembryonic antigen (CEA) levels, CEA tissue staining and histology of small cell cancer of the lung (SCCL). Proc Am Assoc Cancer Res and ASCO 22:345, 1981.
4. GROPP C, HAVERMANN K, and LEHMANN F-G. Carcinoembryonic antigen and ferritin in patients with lung cancer before and during therapy. Cancer 42:2802–2808, 1978.
5. MALKIN A, MALKIN DG, EVANS WK, ET AL. Prognostic value of plasma carcinoembryonic (CEA) determinations in small cell lung cancer (SCLC). Am Assoc Clin Res 185, 1981.
6. PAONE JF, KARDANA A, ROGERS GT, ET AL. Preoperative carcinoembryonic antigen levels correlated with post operative pathological staging in bronchial carcinoma. Thorax 35:920–924, 1980.
7. GROPP C, HAVEMANN K, and SCHEUER A. The use of carcinoembryonic antigen and peptide hormones to stage and monitor patients with lung cancer. Int J Radiat Oncol Biol Phys 6:1047–1053, 1980.
8. Carcinoembryonic antigen: its role as a marker in the management of cancer. National Institutes of Health Consensus Development Conference statement. Cancer Res 41:2017–2018, 1981.
9. COHEN MH, MAKUCH R, JOHNSTON-EARLY A, ET AL. Laboratory parameters as an alternative to performance status in prognostic stratification of patients with small cell lung cancer. Cancer Treat Rep 65:187–195, 1981.
10. SCHNEIDER R, SEIBERT K, PASSE S, ET AL. Prognostic significance of serum LDH in malignant lymphoma. Proc Am Assoc Cancer Res and ASCO 20:337, 1979.
11. ARSENEAU JC, CANELLOS GP, BANKS PM, ET AL. American Burkitt's lymphoma: a clinicopathologic study of 30 cases. Clinical factors relating to prolonged survival. Am J Med 58:314–321, 1975.
12. GRECO FA, SISMANI A, HANDE K, ET AL. Plasma calcitonin and antidiuretic hormone (ADH) as markers in small cell lung cancer. Proc Am Assoc Cancer Res and ASCO 22:342, 1981.
13. ABELOFF MD, TRUMP DL, and BAYLIN SB. Ectopic adrenocorticotrophic (ACTH) syndrome and small cell carcinoma of the lung—assessment of clinical implications in patients on combination chemotherapy. Cancer 48:1082–1087, 1981.
14. GROPP C, HAVEMANN K, and SCHEURER A. Ectopic hormones in lung cancer patients at diagnosis and during therapy. Cancer

46:347-354, 1980.
15. PARSONS RG, LONGMIRE RL, HOCH SO, ET AL. A clinical evaluation of serum C3DP levels in individuals with malignant diseases. Cancer Res 37:692-695, 1977.
16. PARSONS RG, HOCH JA, and LONGMIRE RL. A correlation of serum C3DP levels with chemotherapeutic management of cancer patients. Cancer 41:2099-2106, 1978.
17. TAPIA FJ, MARANGOS PJ, and POLAK JM, ET AL. Neuron-specific enolase is produced by neuroendocrine tumors. Lancet 1(8224): 808-811, 1981.
18. MARANGOS PJ, ZIS AP, CLARK RL, ET AL. Neuronal, non-neuronal and hybrid forms of enolase in the brain: structural, immunological and functional comparisons. Brain Res 150:117-133, 1978.
19. CARNEY DN, MARANGOS PJ, IHDE DC, ET AL. Serum neuron-specific enolase: a marker for disease extent and response to therapy of small-cell lung cancer. Lancet 1:583-585, 1982.
20. AISNER J, WHITACRE M, VAN ECHO DA, ET AL. Doxorubicin, cyclophosphamide and VP15-213 (ACE) in the treatment of small cell lung cancer. Cancer Chemother Pharmacol 7:187-193, 1982.
21. FUKS JZ, VAN ECHO DA, AISNER J, ET AL. Cyclophosphamide (C), adriamycin (A) and etoposide (E) with or without cis-platinum (P) (CAEP vs. CAE) in advanced non-small cell lung cancer (NSCLC). Proc ASCO 1:145, 1982.
22. GERAD H, VAN ECHO DA, OSTROW S, ET AL. Adriamycin, cytoxan and whole body hyperthermia for soft tissue sarcoma. Proc ASCO 1:175, 1982.
23. PARMA AM, MARANGOS PJ, and GOODWIN FK. A more sensitive radioimmunoassay for neuron-specific enolase suitable for cerebrospinal fluid determinations. J Neurochem 36:1093-1096, 1981.
24. SNEDECOR GW, and COCHRAN WC. Statistical Methods. Ames, IA, 6th ed, Iowa State University Press, 1973, pp 193-195.
25. CONLON JC, WALTER AL, and TUBBESING FH. Test and Evaluation of System Reliability, Availability and Maintainability—a Primer. Washington, DC, Office of the Director of Defense—Test and Evaluation, 2nd ed, pp B-51-B-60.
26. GOLD P, and FREEDMAN SO. Demonstration of tumor-specific antigens in human colonic carcinomata by immunological tolerance and absorption techniques. J Exp Med 121:439-462, 1965.
27. STOKES TC, STEVENS JF, LONG P, ET AL. Preoperative carcinoembryonic antigen and survival after resection of lung cancer. Br J Dis Chest 74:390-394, 1980.
28. PARSONS RG, HOCH JA, LONGMIRE RL, ET AL. Serum DNA-binding proteins. *In* Biological Markers of Neoplasia: Basic and Applied Aspects (Ruddon RW, ed). 1978.
29. HANSEN M, HANSEN H, HIRSCH F, ET AL. Hormonal polypeptides and amine metabolites in small cell carcinoma of the lung, with special reference to stage and subtypes. Cancer 45:1432-1437, 1980.
30. HANSEN M, HAMMER M, and HUMMER L. Diagnostic and therapeutic implications of ectopic hormone production in small cell carcinoma of the lung. Thorax 35:101-106, 1980.
31. GILL PG, BRIS R, and HUXTABLE R. Isoenzymes of lactate dehydrogenase (LD) in the serum of patients with malignant disease. UICC Conference in Clinical Oncology 131, 1981.
32. FRIEDMAN A, VUGRIN D, and GOLBEY RB. Prognostic significance of serum tumor biomarkers, alpha-fetoprotein (AFP) beta subunit of human chorionic gonadotrophin (bHCG), and lactate dehydrogenase (LDH) in nonseminomatous germ cell tumors. Proc ASCO 21:323, 1980.
33. VUGRIN D, FRIEDMAN A, SCHWARTZ M, ET AL. Serum lactate dehydrogenase (LDH) isoenzyme 1 (LDH-1) and 1 (LDH-2) as tumor marker in germ cell tumors. Proc Am Assoc Cancer Res and ASCO 21:177, 1980.
34. MOODY TW, PERT CB, GAZDAR AF, ET AL. High levels of intracellular bombesin characterize human small cell lung carcinoma. Science 214:1246-1248, 1981.
35. ERISMAN MD, LINNOILA RI, HERNANDEZ O, ET AL. Human lung small-cell carcinoma contains bombesin. Proc Natl Acad Sci USA 79:2379-2383, 1982.
36. WOOD SM, WOOD JR, GHATEI MA, ET AL. Bombesin, somatostatin and neurotensin-like immunoreactivity in bronchial carcinoma. J Clin Endocrinol Metab 53:1310-1312, 1981.

Blood Leakage and Melphalan Leakage From the Perfusion Circuit During Regional Hyperthermic Perfusion for Malignant Melanoma[1,2]

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In regional hyperthermic perfusion with melphalan for patients with malignant melanoma of the leg, plasma leakage between the perfusion circuit and the systemic circulation was 4–7 ml · min^{-1}. The melphalan concentration in the perfusate was biphasic, with half-lives of 8–12 mins for the initial phase and 19–28 mins for the second phase, after the first dose. After a second dose, the corresponding values were 11–13 and 26–34 mins. The highest concentration in general circulation was 0.38 μg · ml^{-1}. [Cancer Treat Rep 68:867–872, 1984]

Hyperthermic perfusion for malignant melanoma has been used for > 10 years (1,2). The indication has been both for an adjuvant purpose after primary surgery in patients with poor prognosis and for tumor control or palliation in patients with recurrences or metastases restricted to one extremity (3).

Although rather good results have been reported in several studies (3), the technique has not yet been generally accepted. The reasons for this may be that the type of cytostatic drug, temperature level, and duration of perfusion have not been firmly established, and further, that no randomized study has as yet been reported.

The technique is based on isolation of the tumor-bearing limb, which is perfused with a high concentration of an anticancer drug at a temperature of 40.5°C–41.5°C (2–4). The value of hyperthermia in malignant tumor treatment is verified in experimental and clinical work (4–8). The drug most widely used is melphalan (Alkeran). The rationale behind the use of this drug comes from experimental studies in rats with transplanted melanoma. Phenyalanine would carry the alkylating group into the melanin metabolism of the melanoma cells (9).

The ultimate aim of the technique is to avoid systemic toxicity by maintaining the drug within the extremity, without leakage to the rest of the body. Leakage studies have been performed using ^{131}I-albumin (10); the leakage was 10%–30% during 1 hour of perfusion. The aim of the present study was to establish the leakage of blood and melphalan and to determine the half-life of the drug in the hyperthermic circuit.

MATERIALS AND METHODS

Patients and Perfusion Technique

Nine patients were studied, all of whom had melanoma in the leg. Blood leakage was studied in five patients and melphalan leakage was studied in four others.

Under general anesthesia, thermocouples for continuous monitoring of the temperature were inserted im, sc, and epicutaneously on the leg to be perfused. Thermal blankets with a temperature of 38°C were wrapped around the extremity to avoid heat loss. The artery to the extremity and the vein from it were isolated, and catheters were inserted which were connected to a pump, a membrane oxygenator, and a heater. An Esmarck tourniquet was tied as firmly as possible around the extremity proximal to the isolated vessels. When a blood flow of 10–15 ml · kg^{-1} of body weight · min^{-1} had been established and the temperature of the extremity had been increased to 38°C, melphalan (0.45 mg · kg^{-1} of body weight) was added to the circuit. This same dose of melphalan was again added to the perfusion circuit 1 hour later. The dose of melphalan was based on previous clinical studies in which the drug caused the least local side effects (3). An attempt was made to maintain the temperature between 40.0°C and 41.0°C. We also attempted to keep the blood pressure in the perfusion circuit below the mean systemic arterial blood pressure (and the blood flow between 10 and 16 ml · kg^{-1} of body weight · min^{-1}). After 2 hours of perfusion, the circuit

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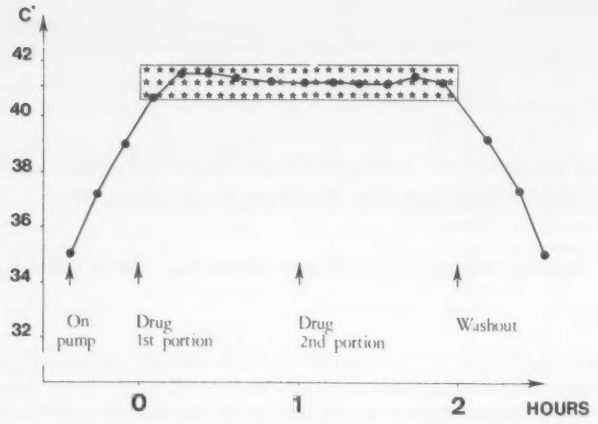

FIGURE 1.—Temperature curve for regional hyperthermic perfusion.

was disconnected and the extremity was rinsed with Ringer's solution (fig 1).

Blood Leakage Studies

Leakage from the perfusion circuit to the general circulation was studied in three leg perfusions. After a good perfusion had been established, ^{125}I-albumin (about 0.7 MBq) was added to the perfusion fluid. Blood samples were drawn at regular intervals from the perfusion circuit and the patient. After centrifugation, ^{125}I activity was measured in 2-ml plasma samples. The volume of the perfusate, ie, "plasma" in the perfusion circuit including the extremity, was calculated using the conventional indicator dilution principle for plasma volume, after extrapolating the plasma concentration curve to zero time (when ^{125}I-albumin was added). The plasma volume in the rest of the patients was assumed to be 45 ml · kg^{-1}.

In two patients, we studied the leakage from the general circulation to the perfusion circuit. ^{125}I-albumin was injected iv in the patients and serial blood samples were drawn from the general circulation and the leg circuit, as mentioned above. The plasma volume was measured in the same way as described above for plasma volume in the perfusion circuit. In these two patients, the plasma volume in the perfusion circuit was assumed to be 800 and 1000 ml, depending on the size of the leg.

Values for blood volume were obtained from plasma volume values using the appropriate hematocrit value.

To facilitate comparison between different patients, all of the concentrations given in figures 2 and 3 were normalized to a theoretical equilibrium concentration, set at 100%. Equilibrium concentration was defined as the injected amount of ^{125}I-albumin diluted in the combined plasma volumes of the perfusion circuit and the patient.

For calculations of leakage, two factors had to be ignored. First, the leakage of albumin from plasma to tissues, and second, the possibility of unequal leakage from leg to patient and vice versa.

Net leakage of albumin was calculated as the difference between the injected amount of albumin and that remaining after perfusion, assuming constant volumes of plasma in the leg and the patient. Net leakage was expressed as percent of the given dose.

Leakage of blood was calculated from the total amount of ^{125}I-albumin lost from the perfusate or the patient's plasma (and appearing in the patient's plasma or the perfusate, respectively). The total amount of albumin was divided by the average difference in albumin concentration between the perfusate and the patient's plasma. It was assumed that the leakage from leg to patient was balanced by an equal leakage from patient to leg. This assumption is supported by the absence of signs of changes in volume of perfusate during the 2 hours of perfusion. Blood or other fluids were not given to the patient or added to the perfusion fluid during perfusion.

Melphalan Leakage

Blood samples were taken from the perfusion circuit and from the general circulation at established intervals in four lower extremity perfusions. The samples were analyzed for melphalan according to the methods described by Chang et al (11). The results were evaluated using a standard curve prepared by adding known amounts of melphalan to plasma. The relative standard deviation was ± 5% at the 5-40-μg/ml level. The degradation of melphalan in vitro was studied in whole blood at 37.0°C, 40.0°C, and 41.5°C.

RESULTS

The concentration of ^{125}I-albumin in the perfusate and in the general circulation after adding the isotope to the

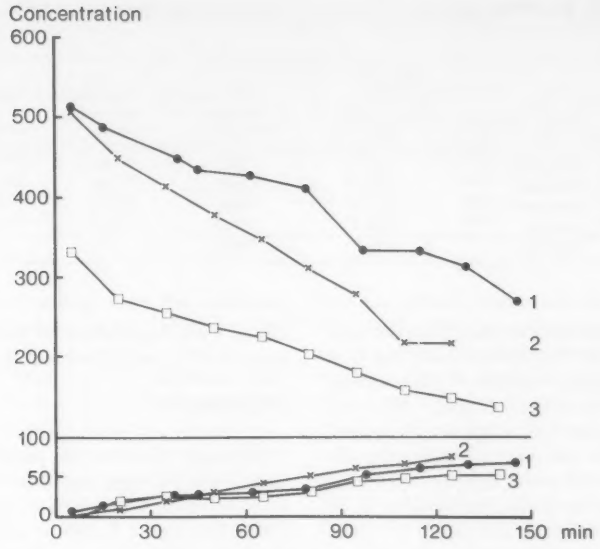

FIGURE 2.—Concentration of ^{125}I-albumin in perfusion fluid (top) and in general circulation (bottom) in Patients 1-3. Albumin was injected into the perfusion system. Concentration values are given as % of the calculated equilibrium concentration (horizontal line at 100; *see* Materials and Methods section).

circuit is illustrated in figure 2. There was a decrease in the concentration in the perfusate and an increase in the general circulation, but equilibrium was not obtained within the time studied. The disappearance curves were somewhat irregular, probably due to varying leakage conditions. If the disappearance curves were assumed to be exponential, half-lives of about 160, 100, and 100 mins were obtained in Patients 1, 2, and 3, respectively. The results when ^{125}I-albumin was added to the general circulation are shown in figure 3. The concentration gradient between the general circulation and the perfusate was much smaller with injection in the patient, because of the larger initial dilution of the albumin. This difference between the two types of experiment is also apparent at the end of perfusion.

Table 1 shows values for net leakage of albumin and blood. When albumin was injected into the perfusion fluid (Patients 1-3), 39%-60% appeared in the patient during 2 hours of perfusion. If albumin was injected into the systemic circulation (Patients 4 and 5), a smaller percentage leaked. This may to a large extent be explained by the smaller concentration difference between the patient and the leg (fig 3), which is due to the larger primary dilution volume of the injected albumin.

The melphalan concentration in the perfusate and estimated regression lines are shown on a logarithmic scale

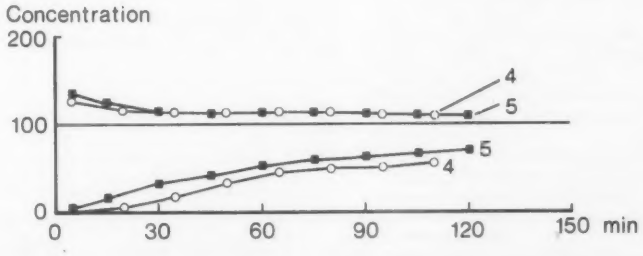

FIGURE 3.—Concentration of ^{125}I-albumin in general circulation (top) and in perfusion fluid (bottom) for Patients 1 and 2. Albumin was injected iv in the patient.

TABLE 1.—Leakage of ^{125}I-albumin from perfusion circuit to general circulation in 3 patients and vice versa in 2 patients

Patient No.	Injection site	Perfusion circuit plasma volume (ml)	Plasma volume (ml)	Net leakage of albumin (%)	Leakage of blood (ml/min)
1	Perfusate	800	3000	50	3.9
2	Perfusate	800	3200	60	6.1
3	Perfusate	1100	3000	39	6.7
4	Patient	800	3300	14	4.8
5	Patient	1000	3000	17	6.7

in figure 4. Declining concentrations were observed with a biphasic elimination curve after about 5 mins (fig 4). It is readily seen that melphalan disappears more rapidly from the perfusion fluid than does albumin. After the first dose, the half-life was 8-12 mins for the initial phase and 19-28 mins for the second phase; after the second dose, the half-life was 11-13 mins for the initial phase and 26-34 mins for the second phase (table 2). In one perfusion (No. 1), a linear elimination curve was found after the second administration, with a half-life of 27 mins. The highest concentration of melphalan in the general circulation was 0.38 $\mu g \cdot ml^{-1}$. The half-life for the in vitro degradation of melphalan in blood was 94 mins at 37°C and 59 mins at 41.5°C (table 3).

DISCUSSION

Although hyperthermic perfusion for malignant melanoma gives a high remission rate of manifest cancer (10% total and 70% partial remission) and has limited side effects (4), it should be possible to further improve these results by increasing the amount of melphalan, testing new drugs, increasing the temperature, etc. Further experiments should be planned from a detailed

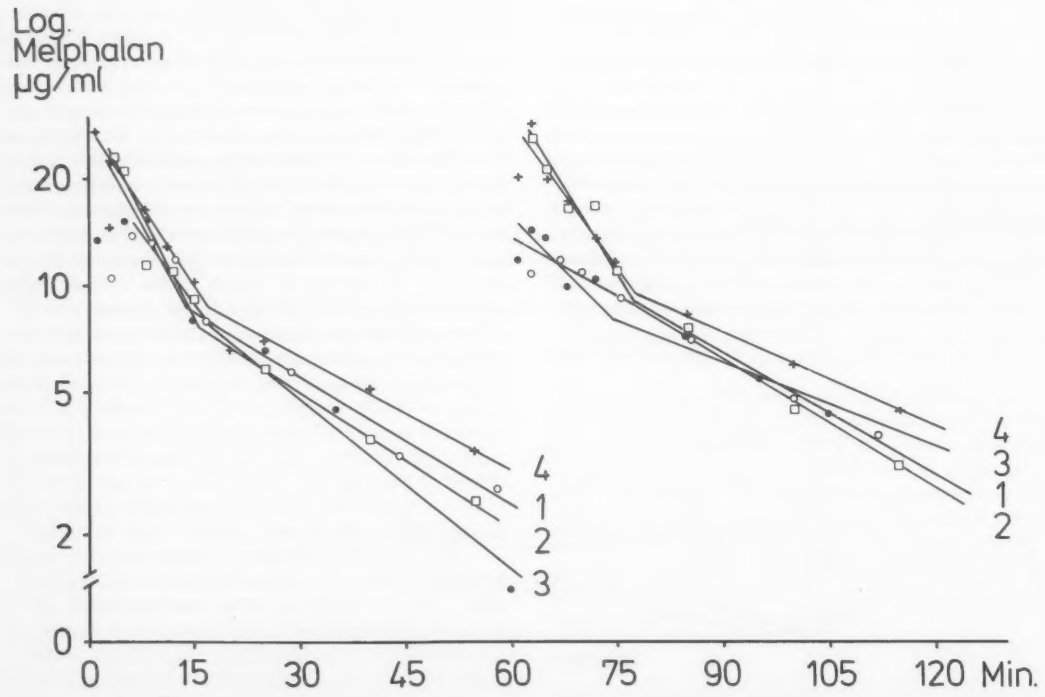

FIGURE 4.—Concentration of melphalan in the perfusion circuit in Patients 1-4.

TABLE 2.—Half-lives and maximum concentrations for melphalan in regional hyperthermic perfusion

Patient No.	Dose of melphalan (%)		Half-life (mins)				Maximum concentration ($\mu g \cdot ml^{-1}$)	
			1st dose		2nd dose			
	1st	2nd	1st phase	2nd phase	1st phase	2nd phase	Perfusion circuit	General circulation
1	25.0	25.0	11.5	26.0	27.0	27.0	13.8	0.09
2	35.0	50.0	8.0	24.0	13.0	25.5	26.0	0.38
3	35.0	35.0	8.5	18.5	12.5	29.0	23.5	0.35
4	27.5	27.5	10.0	28.0	11.0	34.0	28.7	0.31

knowledge of the degree of leakage from the perfusion circuit to the general circulation and the kinetics of the drug in the circuit.

An unexpectedly large leakage of the perfusate from the lower extremities to the circulation was found, with 40%-60% of the albumin being found in the patient. Under the assumptions made, this corresponds to blood flow values of 4-7 ml/min, probably going in both directions between the leg and the patient. These flow values appear small at first sight, at least in comparison with the leg perfusion blood flow of 700-1100 ml/min. During a 2-hour period, however, these leakage flow values correspond to an exchange of a total of 480-840 ml of blood, ie, more than one-half of the entire volume of perfusate.

Further improvement of the isolation technique may reduce leakage somewhat. The limiting factor is probably leakage through the bone marrow of the femur, although leakage through other deep vessels in the upper thigh and hip region may also be involved. Further studies should also focus attention on accurate measurements of arterial and venous pressures in the perfused leg and the patient. The pressure in the feeding artery of the leg must be measured in such a manner that the effect of resistance in the perfusion cannula is eliminated. In addition to this, the direction and magnitude of the blood flow between the leg and the patient must be measured by simultaneous labeling of perfusate and patient blood.

Melphalan had a biphasic elimination curve, with an initial half-life of about 10 mins and a second half-life of 30 mins, which may indicate that administration of the drug at 30-min intervals would give a more evenly distributed concentration in the perfusion circuit.

In most of the perfusions, an initially more rapid elimination curve could be distinguished. This part of the curve is probably due to distribution of melphalan from the blood vessels to extracellular tissue. It can furthermore be noted that the terminal elimination of melphalan in the perfusion circuit is considerably faster than the rate of the chemical degradation in whole blood (table 3), which might reflect leakage of melphalan from the circuit to the general circulation. Albumin disappears, however, at a much slower rate than melphalan. This may be due to back-leakage of albumin from the patient. Melphalan, on the other hand, is not likely to return to the leg since melphalan appearing in the general circulation is likely to disappear quickly by distribution to the large extracellular space.

The peak plasma concentration of melphalan was about 3-5 $\mu g \cdot ml^{-1}$ and the terminal elimination half-life was 86.5 ± 48.8 mins after systemic administration at 37°C of 0.6 $mg \cdot kg^{-1}$ of body weight (12). This might indicate that the leakage of melphalan during regional hyperthermic perfusion is not a limiting factor for increasing the amount of drug. More probable restricting factors are the combined effects of the drug and hyperthermia on, for example, nerves and muscles in the extremity.

TABLE 3.—Influence of temperature on the stability of melphalan in blood*

Temperature	Half-life (mins)†
37.0°C	94
40.0°C	64
41.5°C	59

* Concentration of melphalan: 40 $\mu g/ml$.
† Terminal elimination phase (n = 10).

REFERENCES

1. CAVALIERE R, CIOGATTO EG, GIOVANELLA BC, ET AL. Selective heat sensitivity of cancer cells. Cancer 20:1351-1381, 1967.
2. STEHLIN JS, JR. Hyperthermic perfusion with chemotherapy for cancers of the extremities. Surg Gynecol Obstet 129:305-308, 1969.
3. HAFSTRÖM LO, and JÖNSSON P-E. Regional perfusion with anticancer drugs for treatment of malignant tumors. *In* Tumor Blood Circulation (Petersson H-I, ed). Boca Raton, FL, CRC Press, 1980, pp 217-222.
4. STEHLIN JS, JR, GIOVANELLA BC, DE IPOLYI PP, ET AL. Eleven years' experience with hyperthermic perfusion for melanoma. World J Surg 3:305-307, 1979.
5. HAFSTRÖM LO, and JÖNSSON P-E. Hyperthermic perfusion of recurrent malignant melanoma on the extremities. Acta Chir Scand 146:313-318, 1980.
6. STEHLIN JS, JR, GIOVANELLA BC, DE IPOLYI PP, ET AL. Results of hyperthermic perfusion for melanoma of the extremities. Surg Gynecol Obstet 140:338-348, 1975.
7. CRILE G, JR. The effects of heat and radiation on cancers. Cancer Res 23:372-380, 1963.
8. OVERGAARD K, and OVERGAARD J. Investigation on the possibility of a thermic tumour therapy-short-wave treatment of a transplanted isologous mouse mammary carcinoma. Eur J Cancer 8:65-78, 1972.

9. LUCK JM. Action of p-DI (2-chloro-ethyl)-amino-L-phenylalanine on Harding-Passey mouse melanoma. Science 123:984-985, 1956.
10. STEHLIN JS, JR, CLARK RL, JR, and DEWEY WC. Continuous monitoring of leakage during perfusion. Arch Surg 83:943-949, 1960.
11. CHANG SY, ALBERTS DS, MELNICK IR, ET AL. High pressure liquid chromatographic analysis of melphalan in plasma. J Pharmacol Sci 67:679-681, 1978.
12. ALBERTS DS, CHANG SY, CHEN HG, ET AL. Kinetics of intravenous melphalan. Clin Pharmacol Ther 26:73-80, 1979.

Association Between Malnutrition and Caloric Intake, Emesis, Psychological Depression, Glucose Taste, and Tumor Mass[1,2]

Eduardo Bruera,* Silvia Carraro, Enrique Roca, Liliana Cedaro, and Reinaldo Chacón[3]

A nutritional evaluation was performed in 72 patients with advanced solid tumors receiving chemotherapy: 35 patients (49%) were considered malnourished and 37 (51%) were considered normal. The malnourished group showed a significantly lower caloric intake than the normal group (1214 ± 360 vs 2088 ± 607; $P < 0.001$) and a higher incidence of severe depression (16 of 27 patients [59%] vs six of 30 [20%]; $P = 0.026$). No significant difference was found between the normal and malnourished patients in reference to emesis, glucose tasting threshold, or tumor burden. [Cancer Treat Rep 68:873–876, 1984]

Malnutrition is one of the most commonly described complications in patients with advanced solid tumors. It has been found to occur in 40%–70% of patients (1–3) and cachexia accounts for 20%–25% of cancer deaths (4). In different studies malnutrition has been attributed to decreased caloric intake (5), malabsorption (6), or increased caloric expenditure by the host (7). However, the influence of each factor remains controversial. The purpose of this prospective trial was to establish the relationship between nutritional status and caloric intake, emesis, psychological depression, decreased glucose taste, Van de Kamer's test, and tumor burden in 72 patients with advanced solid tumors receiving chemotherapy.

METHODS

From November 1981 to July 1982, 72 patients with advanced solid tumors receiving chemotherapy (CT) were admitted to our protocol. Patient characteristics are described in table 1. All of the patients were treated on an outpatient basis and had a performance status (Eastern Cooperative Oncology Group) of ⩽ 2. All of the patients had received at least three courses of CT before being admitted to the protocol; none had received irradiation to the head and neck and none were being irradiated. All patients were free from brain metastases or intestinal obstruction.

To determine the nutritional status, five measurements were made: triceps skinfold, arm muscular circumference, albuminemia, creatinine/height index, and actual/habitual weight. All of the results were expressed as a percentage of the standard value for each test, and the five resultant values were averaged. When the resultant percentage was > 90%, the patient was considered normal; when it was between 80% and 90%, he was considered moderately malnourished; and when it was < 80%, he was considered severely malnourished (table 2). To determine daily caloric and protein intake, all of the patients were interviewed by specially trained physicians (E. Bruera and S. Carraro), who collected a single 7-day diet-recall questionnaire. Ten randomized patients were interviewed blindly by two different physicians. Their results showed a good degree of correlation ($R = 0.83$; $P < 0.02$). All of the patients were interviewed between 24 and 48 hours before administration of CT. Caloric balance was determined by subtracting the daily caloric intake from the daily caloric requirements (estimated by Mayo Clinic tables).

The incidence and the intensity of psychological depression were evaluated by Hamilton's psychometric test (8). We chose this test because of its simplicity and reliability in the assessment of different types of depressive illness (9–11). The test was applied only to the last 57 patients, because it was introduced into the protocol after the first 15 patients had already been evaluated. Score values > 5 and > 20 were considered positive for depression and severe depression, respectively. All of the evaluations were performed by specially trained physicians (E. Roca and L. Cedaro).

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TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	72
Mean age in yrs: 53 ± 12	
Sex	
Female	45
Male	27
Primary localization	
Breast	26 (36)
Lung	9 (12.5)
Stomach	8 (11)
Colon	7 (10)
Ovary	7 (10)
Soft tissue sarcoma	4 (5.5)
Kidney	3 (4)
Gallbladder	3 (4)
Prostate	2 (3)
Testis	2 (3)
Melanoma	1 (1)
Total	72 (100)
Cytostatic scheme*	
C + A + P	7 (10)
A + V	20 (28)
A + C	16 (22
C + M + F	6 (8.5)
B + V + P	2 (3)
F + A + MC	10 (14)
A + D	1 (1)
F-HD + A	4 (5.5)
F + ME	3 (4)
C	3 (4)
Total	72 (100)

*C = cyclophosphamide, A = doxorubicin, P = cisplatin, V = vinblastine, M = methotrexate, F = 5-FU, B = bleomycin, MC = mitomycin, HD = high dose, ME = semustine, and D = dacarbazine. Chemotherapy was administered iv. Dosage was the usual for solid tumors.

TABLE 2.—Results of nutritional evaluation

Nutritional status*	No. of patients (%)
Normal	37 (51)
Moderately malnourished	16 (22)
Severely malnourished	19 (27)
	72 (100)

*See the Methods section for definitions of nutritional status and description of evaluation.

To determine the glucose tasting threshold, progressive concentrations from an initial 5-mg/ml (28 mmols/L) glucose solution were used. A randomized sample of 36 patients (18 normal and 18 malnourished) and a control group of 18 healthy adults were tested. All of the patients were tested late in the morning, at least 2 hours after they had eaten or smoked. Patients were asked to taste a baseline 28-mmol/L glucose solution; whenever no taste was recognized the concentration was increased in 11-mmol/L intervals until sweet taste was perceived.

Patients who reported any change in evacuatory rhythm underwent a Van de Kamer's test (12) to detect malabsorption of lipids.

To estimate the clinical tumor mass, scores of 0–4 were used to describe the number and size of all metastasic lesions: 0 = no lesions; 1 = one lesion < 5 cm in any diameter; 2 = one lesion > 5 cm in any diameter or up to two lesions; 3 = between three and five lesions; and 4 = more than five lesions.

After completing each CT cycle, every patient answered a questionnaire about the number and duration of vomiting episodes and the intensity of nausea. To evaluate the intensity of nausea, scores of 0–4 were based on the patients' symptoms and their ability to receive solid or liquid diets. The scoring and quantitation of vomiting were done by the investigators and have been previously described by our group (13).

Statistical analysis was performed by Student's t test for continuous variables and by chi-square test for discrete variables. Whenever a sample was very small or the results approached the significance levels, Fisher's exact test was applied.

RESULTS

The incidence of malnutrition according to our method is shown in table 2. Daily caloric and protein intake is shown in table 3. Van de Kamer's test was positive in two cases (3%).

Although Hamilton's test was positive in 24 of 27 (88%) normal patients versus 24 of 30 (85%) malnourished patients, the incidence of severe depression was 20% (six of 30 patients) in the normal group and 59% (16 of 27) in the malnourished group ($P = 0.026$). Twenty-two of 36 patients (61%) in the patient group had glucose threshold > 60 mmols/L versus one of 18 (5%) in the normal control group ($P < 0.001$), but no significant difference was found between normal (ten of 18, 55%) and malnourished (12 of 18, 66%) patients. No association was found between malnutrition and tumor mass (table 3).

DISCUSSION

Our findings in reference to malnutrition incidence are similar to those from other groups (1) and suggest that a reliable evaluation method was used. The existence of a lower caloric and protein intake and a negative caloric balance in the malnourished group suggests reduced intake as a major cause of malnutrition. The lack of a significant association between malnutrition and emesis, tumor mass, Van de Kamer's test, and glucose recognition test can never be considered proof that such association does not exist, particularly when tested in a small number of patients. However, our data suggest that they were not major causes of malnutrition in our patients. A high incidence of depression was found in both normal and malnourished patients. However, severe depression

TABLE 3.—Summary of different test results, according to nutritional status

Parameter	Normal group (37 patients)	Malnourished group (35 patients)	*P* value*
Mean caloric intake (calories)	2088 ± 607	1214 ± 360	< 0.001
Mean caloric balance (calories)	+401 ± 175	-482 ± 340	< 0.02
Mean protein intake (g)	91 ± 14	58 ± 15	< 0.001
Cytostatic CT-induced emesis†	22/37 (59)	21/35 (60)	NS
Mean duration of nausea and vomiting (hrs)	49 ± 15	43 ± 12	NS
Depression†	24/27 (88)	25/30 (85)	NS
Severe depression†	6/30 (20)	16/27 (59)	< 0.026
Tumor mass, grade 3-4†	21/37 (57)	22/35 (63)	NS
Decreased glucose taste†	10/18 (55)	12/18 (66)	NS
Abnormal Van de Kamer's test†,‡	1/23 (4.3)	1/28 (3.5)	NS

* NS = not significant.

†Values = No. of patients with condition/No. evaluated (%).

‡This test was performed only in patients who reported any change in evacuatory rhythm during the previous 6 mos.

was significantly higher in the malnourished group. This could be a consequence rather than a cause of malnutrition, or perhaps both could be effects of an undetermined group of factors. However, severe essential depression has been found to cause malnutrition in a large number of patients (14). The association between weight loss and depression has been observed in patients with cancer of the pancreas, in whom the incidence of depression was 63%-75% (15), while the frequency of weight loss prior to CT in large series was 80% (1). Further studies are required to establish the incidence and implications of this association in different solid tumors.

Based on the findings of a higher glucose threshold in our patient group (normal and malnourished), we suggest an increase in the sweetening of their meals in an effort to enhance the food intake by stimulating the ingestion control center (16,17). Although the taste sensation abnormalities had been correlated with reduced caloric intake in previous studies (16), further research by the same investigators suggested that the correlation was less clear-cut, "perhaps because of the multiplicity of factors impacting on the food control center" (18).

A significant association between malnutrition and tumor mass could not be established. This might be attributed to the fact that tumor mass was estimated by too simple a method or that the primary localization was not uniform. However, similar findings have been reported by other investigators (19). Although more prospective studies are needed, low caloric intake and depression should be considered in the evaluation of malnourished cancer patients.

REFERENCES

1. DEWYS W, and KISNER D. Principles of nutritional care of the cancer patient. *In* Principles of Cancer Treatment (Carter S, Glatstein E, and Livingston R, eds). Philadelphia, Lippincott, 1982, vol 24, pp 252-259.
2. SMALE BF, MULLEN J, BUZBY G, ET AL. The efficacy of nutritional assessment and support in cancer surgery. Cancer 47:2375-2381, 1981.
3. KISNER DL. The nutrition of the cancer patient. Cancer Treat Rep 65(suppl 5):1-2, 1981.
4. DONALDSON SS, and LENNON RA. Alterations of nutritional status. Impact of chemotherapy and radiation therapy. Cancer 43:2036-2052, 1979.
5. WARNOLD I, LUNDTHOLM K, and SCHERSTEN T. Energy balance and body composition in cancer patients. Cancer Res 38:1801-1807, 1978.
6. LAWRENCE W, JR. Effects of cancer on nutrition. Impaired organ system effects. Cancer 43(suppl):220-229, 1979.
7. BOZZETI F, PAGNONI A, and DELVECCHIO M. Excessive caloric expenditure as a cause of malnutrition in patients with cancer. Surg Gynecol Obstet 150:229-234, 1980.
8. HAMILTON M. A rating scale for depression. J Neurol Neurosurg Psychiatry 23:56-62, 1960.
9. KLAIBER E, BROVERMAN DM, VOGEL W, ET AL. Estrogen therapy for severe persistent depression in women. Arch Gen Psychiatry 36:550-554, 1979.
10. ENDICOTT J, COHEN J, NEE J, ET AL. Hamilton depression rating scale. Extracted from regular and changed version of the schedule for affective disorders and schizophrenia. Arch Gen Psychiatry 38:1981.
11. VENTAFRIDDA V. Il controllo del dolore. *In* Manuale de Oncologia Medica (Bonadonna G, ed). Milano, Masson, 1981.
12. VAN DE KAMER JH, BAKER HEINICK H, and WELJENS H. Rapid method for the determination of fat in feces. J Biol Chem 177:347, 1949.
13. BRUERA ED, ROCA E, CEDARO L, ET AL. Improved control of chemotherapy-induced emesis by the addition of dexamethasone to metoclopramide in patients resistant to metoclopramide. Cancer Treat Rep 67:381-383, 1983.
14. ADAMS RD. Nervousness, anxiety and depression. *In* Harrison's Principles of Internal Medicine, 8th ed (Thorn WG, Adams RD, Braunwald E, et al, eds). McGraw-Hill Book Co, 1977, chap 14, pp 70-72.
15. MACDONALD JS, GUNDERSON LL, and COHN IJ. Cancer of the pancreas. *In* Principles and Practice of Oncology (DeVita VT, Hellman S, and Rosenberg SA, eds). Philadelphia, Lippincott, 1982, pp 563-585.
16. DEWYS WD. Taste and feeding behavior in patients with cancer (Winick M, ed). New York, Wiley, 1977, vol 15.

17. DEWYS WD, and WALTERS K. Abnormalities of taste sensation in cancer patients. Cancer 36:1888–1896, 1975.
18. DEWYS WD, COSTA G, and HENKIN R. Clinical parameters related to anorexia. Cancer Treat Rep 65(suppl 5):49–52, 1981.
19. COHEN A, HEYMESFIELD S, KUCK K, ET AL. Mechanism of weight loss in hospitalized cancer patients. Clin Res 26:36, 1978.

Sequential Methotrexate and 5-FU in CMFP (Cyclophosphamide, Methotrexate, 5-FU, and Prednisone) Therapy for Breast Cancer[1,2]

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Forty-one women with advanced breast cancer were treated with cyclophosphamide, methotrexate, 5-FU, and prednisone. Methotrexate (200 mg/m²) was given iv 1 hour before administering 5-FU (600 mg/m²). Leucovorin (10 mg orally every 6 hours for six doses) was begun 24 hours after the methotrexate. The response rate was 43%, with 19% of patients having a clinically complete remission. This overall objective response rate is lower than that achieved when this drug combination is given at standard doses of methotrexate and with no interval before 5-FU. Toxicity was primarily related to bone marrow suppression; eight women had wbc counts that fell to < 1500/mm³. [Cancer Treat Rep 68:877–879, 1984]

Methotrexate (MTX) and 5-FU are often used in combination for the treatment of breast cancer (1). Recent biochemical studies have demonstrated synergistic antitumor activity in rodents with Sarcoma 180 (2) and breast cancer (3,4) when MTX was given before 5-FU. This synergistic interaction most likely results from enhanced 5-FU accumulation within the MTX-treated cells. A major consequence of this enhanced intracellular 5-FU accumulation is the formation of 5-FUTP which enters RNA and probably accounts for the synergistic killing of tumor cells that results when MTX precedes 5-FU (5–8).

In a recent phase I study in which MTX (200 mg/m² iv) was given 1 hour before 5-FU (600 mg/m² iv), the serum MTX concentrations just before the 5-FU dose were 5–50 μM. Twenty-four hours later, leucovorin (10 mg/m²) was administered orally. Twenty patients with advanced breast cancer were treated, ten of whom had relapsed while receiving cyclophosphamide and conventional doses of MTX and 5-FU (CMF). There were four complete and 12 partial remissions, with minimal toxicity (9).

Recent Eastern Cooperative Oncology Group (ECOG) protocols comparing CMF to CMF plus prednisone (CMFP) and doxorubicin plus vincristine (AV) have shown that the response rate to CMFP (63%) was superior to that to CMF (52%) or AV (53%). The median duration of response was 8 months (10). The purpose of this current study was to test the effectiveness and toxicity of CMFP in which the MTX was administered at 200 mg/m² 1 hour before 5-FU in patients with newly diagnosed breast cancer.

METHODS

Women with measurable metastatic breast cancer who had received no prior chemotherapy and who gave their written informed consent were entered in this study. Prior radiation therapy was acceptable, provided nontreated lesions existed for measurement. The serum creatinine had to be < 1.5 mg/dl and/or the creatinine clearance had to be > 60 ml/minute.

Drug cycles were repeated every 28 days and consisted of cyclophosphamide (100 mg/m²/day orally at bedtime on Days 1–14), MTX (200 mg/m² iv on Days 1 and 8 before cyclophosphamide), 5-FU (600 mg/m² iv 1 hour after the end of MTX administration), prednisone (40 mg/m² orally at bedtime on Days 1–14), and leucovorin (10 mg/m² orally every 6 hours for six consecutive doses, beginning 24 hours after the MTX dose).

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A complete response (CR) was defined as the complete disappearance of all sites of metastatic disease without development of new lesions lasting for a minimum of 4 weeks; bone metastases must have had normalization of skeletal x-rays. A partial response (PR) was defined as at least a 50% decrease in tumor size lasting for a minimum of 4 weeks, without appearance of new lesions.

RESULTS

Forty-one women with advanced breast cancer were treated between October 1979 and July 1981. Their ages ranged between 29 and 72 years (median, 53). Thirty-four of the women (83%) had had a mastectomy an average of 38 months prior to entry in this study. Fourteen women had received prior hormonal therapy (discontinued at least 1 month before treatment was begun); 20 (50%) had had prior radiation therapy; and six (15%) had been treated with ablative hormonal therapy. Thirteen of the women (32%) had had either a subjective or an objective response to one of these forms of treatment. Thirty-six women (88%) had an estrogen-receptor (ER) assay done on either the primary tumor or a metastatic lesion; 16 women were ER-positive. Eleven women (28%) were premenopausal; 28 (72%) were postmenopausal, either naturally (20 women) or surgically (eight women), with an average of 138 months (11.5 years) since menopause.

Three women died from their breast cancer within 4 weeks of beginning therapy, and one was removed from the study due to severe hematologic toxicity. Sixteen of the remaining 37 patients (43%) achieved objective response (CR or PR); ten women (27%) had progression of disease; six (16%) had no change; and five (14%) had minimal response. CRs were achieved in seven women (19%). CRs began between the eighth and 45th week, with an average onset at the 18th week. The duration of response ranged from 5 to 65 weeks. Nine women (24%) had PR which ranged from 4 to 35 weeks in duration (median, 20).

The worst response was in bone tumors, with only five of 19 evaluable lesions showing any response and six of the 19 progressing. Three of the ten lung lesions responded, but 40% progressed. Liver tumors showed a surprisingly high response rate, with five of seven lesions either completely (three patients) or partially (two patients) responding. All lymph node lesions responded to some extent, with eight of the 11 evaluable sites completely responding.

There was no apparent relationship between menopausal status and response. Response rates seemed related to ER status. Women with negative ER status were almost twice as responsive to therapy as those with positive ER status, as documented by the fact that 18 of 22 women with a primary or metastatic lesion that was ER-negative responded, compared to only five of 14 women with an ER-positive assay.

When looking at remission duration with respect to response, there was a noticeable trend, in that the shorter the previous remission, the more likely the woman was to respond to this form of therapy. Seventy-two percent of those women who had been in remission for $\leq$ 1 year responded; the response rates were 67% for those in remission for 1-2 years, 50% for those in remission for 2-3 years, and only 33% for those in remission for $>$ 3 years. The numbers are quite small, especially in the longer remission-duration categories, but the trend is interesting. There was no relationship between age and the likelihood of a response to this therapy.

The toxicity of this therapy, as noted in previous studies, was primarily hematologic, with 37 of 41 women developing some myelosuppression. The degree of toxicity was graded from 1 to 5 (mild to lethal). By ECOG criteria (11), no women were considered to have died from bone marrow suppression, although eight episodes of neutropenia were considered life-threatening, 11 were severe, eight were moderate, and ten were mild. This hematologic toxicity was much greater than that reported with standard CMFP. Twenty-four patients developed nausea, vomiting, or diarrhea, 12 of them to only a mild degree, seven to a moderate degree, and five to a severe degree. Alopecia occurred in 12 women, with at least two patients losing 75%-100% of their hair. Other toxic effects noted were edema (four women), fatigue (four), stomatitis (three), neurological problems (four), life-threatening decrease in creatinine clearance (one), and an elevation of blood glucose (one). All women developed toxic effects of some kind, although ten of the patients developed only a mild degree of either bone marrow or gastrointestinal complications.

CONCLUSIONS

The use of sequential MTX given 1 hour before 5-FU in the CMFP combination drug program for metastatic breast cancer does not have any advantage over standard-dose, nonsequenced CMFP. The objective response rate of 43% does not represent an improvement over the 63% response rate reported for CMFP at standard doses, and the toxicity appears to be substantially greater than that of standard-dose CMFP. Based on this study, we cannot recommend that this 1-hour MTX-5-FU sequence be used in combination with cyclophosphamide and prednisone. We have recently published results showing an in vitro study of human breast cancer cells (10) and a clinical study (12), both of which showed that a 24-hour interval between MTX and 5-FU is more efficacious in killing the cancer cells and quite safe for patients. However, further studies

with the 24-hour interval and with different drug doses are necessary before it can be assumed that this more prolonged interval is clinically useful.

REFERENCES

1. CANELLOS G, DEVITA VT, GOLD G, ET AL. Combination chemotherapy for advanced breast cancer: response and effect on survival. Ann Intern Med 84:389-392, 1976.
2. BERTINO J, SAWICKI W, LINDQUIST C, ET AL. Schedule-dependent antitumor effects of methotrexate and 5-fluorouracil. Cancer Res 37:327-328, 1977.
3. HEPPNER G, and CALABRESI P. Effect of sequence of administration of methotrexate, leucovorin, and 5-fluorouracil on mammary tumor growth and survival in syngeneic C3H mice. Cancer Res 37:4580-4583, 1977.
4. LEE Y, and KHAWAJA T. Adjuvant postoperative chemotherapy with 5-fluorouracil and methotrexate: effect of schedule of administration on metastases of 13762 mammary adenocarcinoma. J Surg Oncol 9:469-479, 1977.
5. CADMAN EC, DAVIS L, and HEIMER R. Enhanced 5-fluorouracil nucleotide formation following methotrexate: explanation for drug synergism. Science 205:1135-1137, 1979.
6. CADMAN EC, and HEIMER R. The influence of methotrexate pretreatment on 5-fluorouracil metabolism in experimental tumor cells. J Biol Chem 256:1695-1704, 1981.
7. BENZ C, and CADMAN EC. The modulation of 5-fluorouracil metabolism and cytotoxicity by antimetabolite pretreatment in human colorectal adenocarcinoma, HCT-8. Cancer Res 41:994-999, 1981.
8. BENZ C, and CADMAN EC. Optimal scheduling of methotrexate and 5-fluorouracil in human breast cancer. Cancer Res 42:2081-2086, 1982.
9. GEWIRTZ AM, and CADMAN EC. Preliminary report on the efficacy of sequential methotrexate, 5-fluorouracil in advanced breast cancer. Cancer 47:2552-2555, 1981.
10. ECOG Group Agenda: EST-Phase III Combination Chemotherapy With and Without Immunotherapy for Metastatic Breast Cancer. Nov 1977.
11. OKEN M, CREECH R, TORNEY D, ET AL. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Cancer Clin Trials, 1982. In press.
12. BENZ C, SILVERBERG M, and CADMAN E. Use of high-dose oral methotrexate sequenced at 24 hours with 5-FU: a clinical toxicity study. Cancer Treat Rep 67:297-299, 1983.

Phase I–II Study of Aclarubicin for Treatment of Acute Myeloid Leukemia[1,2,3]

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Aclarubicin (ACM) was administered as induction treatment to 40 patients with acute myeloid leukemia (AML) who were either refractory to initial induction chemotherapy or in relapse. Thirty-eight patients with AML, 2–80 years of age (mean ± SE, 35.0 ± 3.2), were evaluated during this study. Seventeen of these patients were given ACM after an unsuccessful attempt had been made to attain a complete remission (CR) with various regimens that included doxorubicin or daunorubicin; this group was considered resistant to these drugs. ACM was administered by rapid iv injection. Thirteen patients received a single course of ACM at a daily dose of 10–30 mg/m² until a maximum total dose of 300 mg/m² was reached or until unacceptable toxicity appeared. Of these patients, two (15%) attained a CR. The other 25 patients were given 10-day courses of ACM at a daily dose of 15 mg/m² with 10-day intervals between courses; courses were repeated until the blast cells were cleared from peripheral blood and bone marrow or until progressive disease became evident. With this regimen, 11 patients (44%) attained a CR. The overall CR rate for the 38 patients was 34%. Total doses necessary to achieve a CR ranged from 150 to 600 mg/m². A CR was attained by six patients who were previously resistant to a regimen containing moderate doses of doxorubicin. The incidence and severity of the toxic effects were related to the dose of ACM administered per course of therapy. The incidence of mucositis, diarrhea, vomiting, and infection in patients who received doses > 150 mg/m²/course was significantly higher than that observed in patients who received a dose of 150 mg/m²/course. In the latter patients, toxicity was within acceptable limits. Alopecia was not observed. Three patients had transient T-wave inversion, and reversible atrial flutter developed in one patient. Our results indicate that ACM is a major new drug for the treatment of AML. [Cancer Treat Rep 68:881–886, 1984]

Aclarubicin (ACM) is one of the antitumor anthracyclines produced by fermentation of *Streptomyces galilaeus* MA-144-M1 (1). It differs from daunorubicin (DNR) and doxorubicin (ADM) in several substitutions on the hydrophobic rings (aklavinone) and in the three hexopyranoses which are attached to aklavinone by glycosidic linkage. In the Ames test, ACM, unlike DNR and ADM, was found to be nonmutagenic (2). The drug exerts a wide spectrum of activity against animal tumors (3–5) and has been shown to be less cardiotoxic than ADM and DNR (6,7).

Initial phase I studies have been reported previously by others (8–10). The toxic effects noted were transient disturbances of hepatic function, mild gastrointestinal toxicity, and dose-dependent myelosuppression. Alopecia was minimal and minor ECG abnormalities were seldom observed. No case of congestive heart failure (CHF) was reported. In these phase I studies, tumor regression was

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observed in a small percent of patients with lymphomas and various carcinomas.

In our own preliminary studies, we observed antitumor activity of ACM in acute lymphocytic leukemia, leukemic lymphoma, and acute myeloid leukemia (AML) (11-13). These early studies have neither fully explored the degree of activity of ACM in AML nor clarified the optimal dose schedule when the drug is used on a daily administration basis. In the present report, we describe a phase I-II study of ACM for AML, using two different treatment modalities. This study was conducted from September 1978 to October 1982.

PATIENTS AND METHODS

Patients with overt AML were eligible for entry in this study either after initial failure to respond to standard induction chemotherapy or upon relapse. Forty patients were entered in this study. Two of these patients were later excluded because of protocol violations: one left the hospital before the treatment was completed and the other was inadvertently given an inappropriate dose (ie, one single injection of ACM at a dose of 150 mg/m^2). The 38 evaluable patients (table 1) were from 2 to 80 years of age (mean ± SE, 35.0 ± 3.2); 23 were male and 15 were female. None of the patients had any history of previous myeloproliferative or myelodysplastic syndromes. All cases were subclassified according to the World Health Organization (WHO) classification of neoplastic hematopoietic diseases (14); cytologic types included 28 cases of acute myelocytic leukemia, one case of acute promyelocytic leukemia, and nine cases of acute myelomonocytic leukemia. All patients had received previous chemotherapy for AML, including DNR or ADM or both. Five patients had shown primary resistance to one to three courses of an intensive regimen combining ADM (50 mg/m^2, Day 1), vincristine (VCR) (1 mg/m^2, Day 2), and cytarabine (ara-C) (160 mg/m^2/day, Days 2-8), and 33 patients were treated with ACM after one to three relapses. Of these 33 patients in relapse, 12 received ACM after an unsuccessful attempt had been made to obtain another remission with various intensive regimens that included ADM or DNR: eight patients had received the same chemotherapy described above, three had been treated with a regimen combining DNR (45 mg/m^2/day, Days 1-3), 6-thioguanine (100 mg/m^2/day, Days 1-5), and ara-C (100 mg/m^2/day, Days 1-5), and one had received DNR alone (25 mg/m^2/day, Days 1-5). Therefore, a total of 17 patients were considered resistant to ADM or DNR. The remaining 21 patients received ACM immediately after the diagnosis of relapse; their status in regard to resistance to ADM or DNR was thus unknown.

ACM was given daily by rapid iv injection, using two therapeutic regimens. In *Regimen 1*, sets of two to four patients were treated with increasing daily doses until a maximum total dose of 300 mg/m^2 was reached or until unacceptable toxicity appeared. The starting daily dose was 10 mg/m^2. Each of the 13 patients treated from September 1978 to March 1980 received a single course of ACM, according to one of the four following schedules: (*a*) two patients received 10 mg/m^2/day, (*b*) four received 15 mg/m^2/day, (*c*) three received 20 mg/m^2/day,

TABLE 1.—Characteristics of the 38 patients with AML treated with ACM

Characteristic	No. of patients		
	Regimen 1	Regimen 2	Total
Age in yrs (range)			
2-10	2	3	5
11-39	5	12	17
40-80	6	10	16
Sex			
Male	10	13	23
Female	3	12	15
Cytologic type			
Acute myelocytic	10	18	28
Acute promyelocytic	—	1	1
Acute myelomonocytic	3	6	9
No. of previous remissions			
0	2	3	5
1	6	18	24
2	4	4	8
3	1	—	1
Prior resistance to DNR or ADM			
Yes*	7	10	17
Unknown	6	15	21

* Patients who received ACM after an unsuccessful attempt had been made to achieve a complete remission with various intensive regimens including ADM or DNR.

and (*d*) four received 30 mg/m²/day. By May 1980, the treatment modality was modified because the toxicity with Regimen 1 was high. In *Regimen 2*, 25 patients received a cyclic treatment with ACM. They were given 10-day courses at a daily dose of 15 mg/m²; the intervals between courses were fixed at 10 days. For these 25 patients, courses of ACM were repeated until the blast cells were cleared from peripheral blood and bone marrow or until progressive disease became evident.

Before entering the trial, all patients were evaluated by physical examination, marrow aspirate, blood cell count, the usual biochemical profile, ECG, and chest roentgenograms. During the treatment, bone marrow smears were examined weekly; blood cell counts were performed daily; and liver function tests, serum electrolytes, BUN, and creatinine were determined at least twice a week. An ECG was performed before each dose of ACM, and QRS voltages were compared according to the method of Minow et al (15). All of the patients received intensive hematologic supportive treatment.

Cancer and Leukemia Group B (CALGB) remission criteria were used (16), and only complete remission (CR) was considered to be significant. After attaining CR, patients received various maintenance chemotherapy regimens. Toxicity was scored according to WHO recommendations (17). Statistical comparisons were performed with the chi-square test.

RESULTS

Response to Therapy

Of the 13 patients subjected to Regimen 1, four received a total dose of 300 mg/m² and nine received lower doses ranging from 150 to 280 mg/m². In this group, CR was attained by two patients (15%) who received a total dose of 300 mg/m². Durations of CR were 6 and 17 weeks (table 2). Of the 25 patients who received Regimen 2, 11 (44%) attained CR. CRs were attained after one course (150 mg/m²) by three patients, after two courses (300 mg/m²) by five, after three courses (450 mg/m²) by one, and after four courses (600 mg/m²) by two. Durations of CR were 5, 8, 9, 10, 22, 26, 30, 30, 34, 38, and 86 weeks. The overall CR rate for 38 patients was 34%. Upon relapse, three of the 11 complete responders to Regimen 2 underwent a second induction treatment with ACM, and two patients again attained CR of 6 and 13 weeks. Furthermore, as indicated in table 3, six patients who had previously shown resistance to a regimen including VCR, ara-C, and moderate doses of ADM attained CR with ACM.

No statistical difference was found in comparisons of the incidence of CR according to age, cytologic type of AML, or number of previous remissions (table 3). Most of the patients had no cytogenetic studies; therefore, the results could not be analyzed for the incidence of CR.

Toxicity

The incidence and severity of the toxic effects were related to the total dose of ACM administered during each course of therapy (table 4). Toxic effects led to discontinuation of therapy in nine patients subjected to Regimen 1, at total doses ranging from 150 to 280 mg/m². In this regimen, only the four patients who received 30 mg/m²/day could receive the total planned dose of 300 mg/m². This was explained by the delayed appearance of toxicity induced by ACM (occurring in most cases after the tenth day of treatment), which only allowed completion of the shorter courses. All patients treated with Regimen 2 received the total planned dose of 150 mg/m²/course.

All patients had granulocytopenia with < 200 granulocytes/mm³; the platelet count was < 30,000/mm³, requiring platelet transfusions. The time required for recovery of myelopoiesis, from the end of treatment, was

TABLE 2.—Response to ACM according to regimen and total dose administered

Total dose (mg/m²)	No. of patients	No. of patients in CR (%)	Duration of CRs in wks*
Regimen 1			
150-200	3	0	—
201-280	6	0	—
300	4	2	6, 17
	13	2 (15)	
Regimen 2			
150	7	3	26, 38, 86
300	11	5	9, 10, 30, 30, 34
450	3	1	22
600	4	2	5, 8
	25	11 (44)	
Regimens 1 and 2	38	13 (34)	

* After attaining a CR, the patients received various maintenance chemotherapy regimens.

TABLE 3.—Response to ACM according to patient characteristics

Characteristic	Response to therapy (No. of patients) CR	Failure	P value*
Age range (yrs)			
2-39	9	13	0.31
40-80	4	12	
Cytologic type			
Acute myelocytic	9	19	0.95
Acute myelomonocytic	3	6	
Acute promyelocytic	1†	0	
No. of previous remissions			
0 or 1	9	20	0.74
2 or 3	4	5	
Prior resistance to DNR or ADM			
Yes‡	6§	11	
Unknown	7	14	

*Statistical comparisons of the incidence of CR with characteristics of patients were performed with the chi-square test.

†Not included in statistical comparisons.

‡Patients who were resistant to previous induction treatment including ADM or DNR (only doses of anthracyclines are given): 13 patients received ADM (50 mg/m^2, Day 1), VCR, and ara-C; 3 were treated with DNR (45 mg/m^2, Days 1-3), 6-thioguanine, and ara-C; and 1 received DNR alone (25 mg/m^2, Days 1-5).

§2 patients were treated with Regimen 1, and 4 with Regimen 2. All patients had previously shown resistance to combined treatment with ADM, VCR, and ara-C.

25-35 days in responders treated with Regimen 1 and 10-15 days in those treated with Regimen 2.

Diarrhea, mucositis, and infection complicating granulocytopenia were the most prominent toxic effects. Nausea and vomiting were less prevalent. The incidence of these toxic effects in the 12 patients who received doses of ACM > 150 mg/m^2/course was significantly higher than that observed in the 26 patients who received a dose of 150 mg/m^2/course (table 4). A transient, mild increase in serum transaminases, mainly SGPT, was observed in four patients. However, hepatic toxicity was difficult to assess, because these four patients were heavily transfused and were receiving concurrent antibiotic therapy which could explain their mild liver dysfunction.

Alopecia did not occur in any of the 14 patients evaluable for hair loss.

As shown in table 5, three patients developed transient inversion of T-wave and one had one episode of atrial flutter. None of these patients had any underlying cardiac disease or hypertension. No significant decrease in the QRS amplitude was observed. CHF did not occur during the observation period.

Local phlebitis at the injection site was not observed and drug extravasation did not occur in any of the patients.

DISCUSSION

The results of this study indicate that ACM induced a high rate of CR (34% overall) in previously treated AML patients. With Regimen 1, the CR rate was 15%. This treatment modality produced a high incidence of severe toxic effects which usually appeared after the tenth day of treatment at total doses > 150 mg/m^2, leading in most cases to discontinuation of therapy. In Regimen 2, we were able to avoid severe toxicity in most of the patients. With the cyclic schedule employed in this regimen, the CR rate was also improved: 44% of the patients attained CR after one to four treatment courses.

From this study, it was not possible to determine the minimal total dose of ACM required for patients to achieve a CR. Some patients attained CR after receiving 150 mg/m^2, which was the lowest total dose administered during the study. However, the cumulative dose required for the majority of the patients to achieve CR was ≥ 300 mg/m^2. We believe that the low response rate obtained with Regimen 1 could be explained in part by the insufficient amount of ACM administered to most patients, because of the major toxic effects induced by the treatment. Regimen 2, a much less toxic schedule, allowed the administration of higher total doses of ACM and, therefore, improved the therapeutic efficacy of the drug.

Yamada et al (18) reported CR rates of 35% in 20 previously untreated patients and 18% in 33 previously treated patients with AML. The schedule of ACM used in their trial consisted of daily administration of 15 mg/m^2 until toxicity developed. Warrell et al (19) administered ACM at doses of 100-120 mg/m^2/day for 2-3 days to 34 patients with acute nonlymphocytic leukemia. Only four (11%) of these patients attained CR.

TABLE 4.—Toxic effects according to dose of ACM/course*

Toxic effect	No. of patients with toxic effects, according to dose/course (mg/m^2)			
	150† (N = 26)	170–200 (N = 2)	201–250 (N = 3)	251–300 (N = 7)
Oral mucositis‡				
1	1	0	0	0
2	5	1	2	4
3	2	0	0	0
4	0	0	1	1
	8	1	3	5
Diarrhea‡				
2	4	1	2	1
3	0	1	0	5
4	0	0	1	1
	4	2	3	7
Nausea/vomiting‡				
1	3	1	1	2
2	2	0	2	4
	5	1	3	6
Infection§				
Nondocumented fever	3	0	1	1
Septicemia	7 (2)	1	2	4
Pneumonitis	1 (1)	0	0	1
Septicemia and pneumonitis	0	1 (1)	0	1
	11	2	3	7
Liver dysfunction	4¶	0	0	0
Renal dysfunction**	—	—	—	—
Alopecia††	—	—	—	—

* Chi-square test showed a highly significant difference when the incidence of mucositis, diarrhea, vomiting, and infection was compared between patients who received 150 mg/m^2/course and patients who received ≥ 170 mg/m^2/course. P values were < 0.02, 10^{-6}, < 0.001, and < 0.01, respectively.

†This group includes 1 patient who received Regimen 1 and all patients treated with Regimen 2.

‡Values = toxicity score according to WHO recommendations (ref 17).

§Values in parentheses denote patients who died of infection.

¶Transient, mild increase of serum transaminases, mainly SGPT (150–200 units/L; normal values, 15–35 units/L).

** No patients had renal dysfunction.

††No alopecia was seen in 143 evaluable patients.

The schedules used in the present study were selected on the basis of experimental data obtained with tumor-bearing mice (3–5,20). The optimal dose and schedule of ACM for treatment of AML are still uncertain. Our results and those obtained by others (18,19,21) suggest that daily administration of small doses of ACM over an extended period is more effective than administration of high doses for a short time. However, our results also indicate that in the case of prolonged treatment with small daily doses, the total amount of ACM per course should not exceed 150 mg/m^2, in order to avoid severe drug-induced toxicity. For these reasons, we recommend for fur-

TABLE 5.—Cardiac toxicity according to cumulative dose of ACM administered (N = 38)

Type of cardiac toxicity	No. of patients	Cumulative dose of ACM at which toxicity appeared (mg/m^2)	Previous amount of DNR and/or ADM (mg/m^2)
Transient inversion of T-wave	3	450, 510, 690	300, 140, 100
Reversible atrial flutter	1	300	290
None	34*		270 (mean)

* The 34 patients without cardiac toxicity received doses of ACM ranging from 150 to 600 mg/m^2 (mean, 310); all had previously been treated with ADM or DNR or both.

ther clinical trials the administration of ACM at a daily dose of 15 mg/m² over 10 days with 10-day intervals between courses.

Moreover, six patients previously resistant to an intensive regimen comprising VCR, ara-C, and moderate doses of ADM attained a CR with ACM. These results support previous clinical studies (18,22,23) which suggest that there is no cross-resistance between ADM or DNR and ACM. Absence of cross-resistance has been experimentally demonstrated in Friend leukemia cell lines (24). However, other studies performed with DNR-resistant L1210 and ADM-resistant P388 leukemias have shown that these cells were also resistant to ACM (3,5).

Dose-related oral mucositis and diarrhea were prominent toxic effects observed during this study. Other investigators (18,19) have reported mucositis in 32%-53% and diarrhea in 12%-26% of patients with acute leukemia treated with ACM.

Alopecia was not observed in the present study. This is in agreement with experimental results obtained in ACM-treated hamsters (6).

In the present study, cardiac toxicity was limited to transient T-wave inversion in three patients and to a single episode of atrial flutter. Other clinical studies have shown that ACM can induce cardiac dysrhythmias and repolarization disturbances (18,19,25,26). CHF attributable to ACM has not been reported thus far. However, an assessment of the risk of chronic anthracycline cardiomyopathy will not be possible until accurate cardiac monitoring is carried out on a much larger number of patients treated with higher cumulative doses of ACM.

We conclude that ACM appears to be a major new drug for the treatment of AML. Its use as a single agent and in combination regimens merits further clinical trials.

REFERENCES

1. OKI T, MATSUZAWA Y, YOSHIMOTO A, ET AL. New antitumor antibiotics, aclacinomycins A and B. Jpn J Antibiot 28:830-834, 1975.
2. UMEZAWA K, SAWAMURA M, MATSUSHIMA T, ET AL. Mutagenicity of aclacinomycin A and daunomycin derivatives. Cancer Res 38:1782-1784, 1978.
3. HORI S, HURANO S, ORI T, ET AL. Antitumor activity of new anthracycline antibiotics, aclacinomycin A and its analogues, and their toxicity. Gan 68:685-690, 1977.
4. OKI T. New anthracycline antibiotics. Jpn J Antibiot Suppl 30:570-584, 1977.
5. OKI T, TAKEUCHI T, OKA S, ET AL. New anthracycline antibiotic aclacinomycin A: experimental studies and correlations with clinical trials. *In* New Drugs in Cancer Chemotherapy (Carter SK, Sakurai Y, and Umezawa H, eds). Berlin-Heidelberg-New York, Springer-Verlag, 1981, pp 21-40.
6. DANTCHEV D, SLIOUSSARTCHOUK V, PAINTRAND M, ET AL. Electron microscopic studies of the heart and light microscopic studies of the skin after treatment of golden hamsters with adriamycin, detorubicin, AD-32, and aclacinomycin. Cancer Treat Rep 63:875-888, 1979.
7. WAKABAYASHI T, OKI T, TONE H, ET AL. A comparative electron microscopic study of aclacinomycin and adriamycin cardiotoxicities in rabbits and hamsters. J Electron Microsc 29:106-118, 1980.
8. FURUE E, KOMITA T, NAKAO I, ET AL. Clinical experiences with aclacinomycin A. *In* Antitumor Antibiotics (Carter SK, Umezawa H, Douros J, et al, eds). Berlin-Heidelberg-New York, Springer Verlag, 1978, pp 241-246.
9. OGAWA M, INAGAKI J, HORIKOSHI N, ET AL. Clinical study of aclacinomycin A. Cancer Treat Rep 63:931-934, 1979.
10. SAKANO T, OKAZAKI N, ISE T, ET AL. Phase I study of aclacinomycin-A. Jpn J Clin Oncol 8:49-53, 1978.
11. MATHÉ G, BAYSSAS M, GOUVEIA J, ET AL. Preliminary results of a phase II trial of aclacinomycin in acute leukaemia and lymphosarcoma. An oncostatic that is rarely cardiotoxic and induces no alopecia. Cancer Chemother Pharmacol 1:259-262, 1978.
12. MATHÉ G, GIL MA, GESCHER F, ET AL. Aclacinomycin A in acute leukaemias and lymphomas. Lancet 2:310-311, 1979.
13. MATHÉ G, DE JAGER R, HULHOVEN R, ET AL. L'aclacinomycine A dans les leucémies aigues et les lymphomes non-hodgkiniens leucémiques. Nouv Presse Med 11:25-28, 1982.
14. MATHÉ G, and RAPPAPORT H. Histological and Cytological Typing of Neoplastic Diseases of Haematopoietic and Lymphoid Tissues. Geneva, World Health Organization, 1976.
15. MINOW RA, BENJAMIN RS, LEE ET, ET AL. QRS voltage change with adriamycin administration. Cancer Treat Rep 62:931-934, 1978.
16. ELLISON RR. Acute myelocytic leukemia. *In* Cancer Medicine (Holland JF, and Frei E, III, eds). Philadelphia, Lea and Febiger, 1973, pp 1199-1234.
17. WHO Handbook for Reporting Results of Cancer Treatment. Geneva, World Health Organization, 1979.
18. YAMADA K, NAKAMURA T, TSURUO T, ET AL. A phase II study of aclacinomycin A in acute leukemia in adults. Cancer Treat Rev 7:177-182, 1980.
19. WARRELL RP, ARLIN ZA, KEMPIN SJ, ET AL. Phase I-II evaluation of a new anthracycline antibiotic, aclacinomycin A, in adults with refractory leukemia. Cancer Treat Rep 66:1619-1623, 1982.
20. NARA N, MIYAMOTO T, HIRASHIMA K, ET AL. Effects of aclacinomycin A on murine leukemia. Blood 60:188-193, 1982.
21. MITROU PS. Aclacinomycin A (ACM) in the treatment of relapsing acute leukaemia. *In* Proceedings of the 13th International Congress of Chemotherapy, Vienna (Umezawa H, Mathé G, and Mitrou PS, eds). Part 211, 1983, pp 45-48.
22. SUZUKI H, KAWASHIMA K, and YAMADA K. Aclacinomycin A, a new anti-leukaemic agent. Lancet 1:870-871, 1979.
23. TAKAHASHI I, HARA M, ADACHI T, ET AL. Treatment of refractory acute leukemia with aclacinomycin A. Acta Med Okayama 34:349-354, 1980.
24. TAPIERO H, FOURCADE A, and BENNOUN M. Comparative uptake of adriamycin, daunorubicin and aclacinomycin in sensitive and resistant Friend leukemia cells. *In* Anthracyclines 1981: Current Status and Future Development (Mathé G, Maral R, and De Jager R, eds). New York, Masson Publication, 1983, pp 95-100.
25. VAN ECHO DA, WHITACRE MY, AISNER J, ET AL. Phase I trial of aclacinomycin A. Cancer Treat Rep 66:1127-1132, 1982.
26. AABO K, MORTENSEN SA, SKOVSGAARD T, ET AL. Intermittent high-dose aclarubicin in patients with advanced cancer: a phase I study with special reference to cardiac toxicity. Cancer Treat Rep 67:281-282, 1983.

Venous Thrombosis as a Side Effect of Tamoxifen Treatment[1]

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We report what we believe to be an infrequent but noteworthy complication of antiestrogen therapy with tamoxifen. Seven patients developed venous thrombosis or pulmonary embolism within 6 months of starting treatment. Guidelines similar to those employed in younger women treated with anovulatory drugs would appear indicated. [Cancer Treat Rep 68:887–889, 1984]

Tamoxifen is an effective antiestrogenic drug. It binds to the cytoplasmic estrogen receptor (ER) and this tamoxifen-ER complex is transported to the nucleus. Tamoxifen prevents replenishment of the cytoplasmic ER. In paradoxical fashion, tamoxifen also has estrogenic properties in rodents and in human breast cancer cell lines in culture (1). Tamoxifen also induces an increase in progesterone receptors in patients with adenocarcinoma of the endometrium (2). This increase in progesterone receptors is clearly an estrogenic effect. In addition, Sabai et al (3) have reported an increase in steroid-binding globulins in patients with breast cancer treated with tamoxifen, which is also an estrogenic effect.

Estrogenic compounds are associated with an increased incidence of thromboembolic disease. We report here seven patients who developed venous disease or pulmonary embolism while receiving tamoxifen therapy for breast cancer.

PATIENTS AND METHODS

Between January 1980 and December 1982, 220 patients started receiving tamoxifen at The Milton S. Hershey Medical Center for treatment of their metastatic breast carcinoma. In this report, we present data on seven of these patients who developed clinical venous disease or nonfatal lung thromboembolism during this period. All patients developed these complications within 6 months of starting tamoxifen therapy.

RESULTS

The characteristics of the seven patients who developed clinical venous disease or pulmonary embolism are presented in table 1. Widespread metastatic disease was present in four of the patients. There was no clinical evidence of recurrent cancer in Patients 3 and 6. In addition, Patient 7 had minimal metastatic disease present in one vertebral body.

Four of the seven patients received combination chemotherapy in addition to tamoxifen. One patient received concomitant aminoglutethimide therapy. This latter drug has not been associated with venous disease or thromboembolism.

DISCUSSION

Tamoxifen has gained widespread usage in the treatment of metastatic breast cancer because of its efficacy and safety. Indeed, it has recently been incorporated into adjuvant therapy protocols.

In this report, we present seven patients who developed clinical venous disease or nonfatal lung embolism within 6 months of starting tamoxifen therapy. In all patients, there was no prior history of similar difficulty. In the literature, there is a suggested association between thrombophlebitis and antiestrogen treatment (4–14). Indeed, in one series of 30 patients treated with tamoxifen, two patients developed "a possibly drug-related superficial phlebitis of the leg" (4).

In 1865, Trousseau (15) first described an association between a mucin-producing gastric carcinoma and recurrent migratory thrombophlebitis. Thus, cancer itself can predispose to venous disease. This fact makes it most difficult to be entirely certain that there is a causative relationship between antiestrogen treatment and a real but low incidence of venous disease. The ab-

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TABLE 1.—Clinical summary

Patient No.	Mastectomy (mo/yr)	Metastases (mo/yr)	Start of tamoxifen therapy (mo/yr)	Chemotherapy	Complications (mo/yr)
1	—	Bone, lung (8/80)	11/81	Mitomycin + lomustine	Phlebitis (12/81)
2	3/80	Bone (8/81)	9/81	—	Phlebitis (12/81)
3	1/81	None	7/81	—	Phlebitis (1/82)
4	3/80	Chest wall (8/81)	2/82	Cyclophosphamide, doxorubicin, vincristine, and prednisone	Pulmonary emboli (4/82)
5	1960	Brain, bone (9/82)	10/82	Cyclophosphamide, methotrexate, 5-FU, and vincristine	Phlebitis, pulmonary embolus (11/82)
6	2/82	None	3/82	Cyclophosphamide, methotrexate, 5-FU, and aminoglutethimide	Phlebitis, pulmonary embolus (6/82)
7	2/82	T9	6/82	—	Superficial phlebitis, leg (7/82)

sence of proven malignancy in Patients 3 and 6 and metastatic disease in only one site in Patient 7 led us to infer that the presence of cancer did not explain the observed events in all of our patients. Chemotherapy itself has been reported to predispose to venous thromboembolic disease. However, three of the patients reported were not receiving any drug treatment.

The manufacturers of tamoxifen have been alerted to this possible side effect. A review of the data from their past and ongoing clinical trials, as well as their marketed drug experience, revealed the following: eight cases of phlebitis, three of thrombophlebitis, and four of thrombosis. Together, these data bases contain 1975 patient cases,[4] an incidence of slightly less than one case per 100 treated patients. This is compared to our experience in which seven cases of phlebitis and/or pulmonary embolus were observed in approximately 220 patients treated with tamoxifen over a 3-year period (3.2%).

The mechanism of this suggested relationship between tamoxifen and venous thromboembolic disease is speculative at present. However, a depression of antithrombin III activity has been reported in ten of 24 patients (42%) treated with tamoxifen (16). It is not known whether this depression of antithrombin III is due to the estrogenic or antiestrogenic effect of tamoxifen.

In conclusion, we wish to emphasize the possible association between antiestrogen therapy with tamoxifen and venous thrombosis in various sites (calf veins and lungs). Although this is a relatively rare event, it may lead to life-threatening disease. Such an association must be suspected in patients who develop venous disease or pulmonary embolism within 6 months of starting tamoxifen therapy. The drug should be discontinued. We feel that guidelines similar to those employed in younger women treated with anovulatory drugs would appear to be indicated.

[4]White HN. Personal communication. Stuart Pharmaceuticals, Division of ICI Americas, Inc, Wilmington, DE.

REFERENCES

1. LIPPMAN M, BOLAN G, and HUFF K. The effects of estrogens and anti-estrogens on hormone responsive cancer in long-term tissue culture. Cancer Res 35:4595-4601, 1976.
2. MORTEL R, LEVY C, WOLFF JP, ET AL. Female sex steroid receptor in postmenopausal endometrial carcinoma and biochemical response to an antiestrogen. Cancer Res 41:1140-1147, 1981.
3. SABAI F, CHEIX F, CLAVEL M, ET AL. Increases in steroid binding globulins induced by tamoxifen in patients with carcinoma of the breast. J Endocrinol 76:219-226, 1978.
4. BEEX L, PIETERS G, SMALS A, ET AL. Tamoxifen versus ethinyl estradiol in the treatment of postmenopausal women with advanced breast cancer. Cancer Treat Rep 65:179-185, 1981.
5. BEEX L, PIETERS G, SMALS A, ET AL. DES versus tamoxifen in advanced breast cancer. N Engl J Med 304:1041-1043, 1981.
6. BOSCH F, ALONSO MUNOZ J, OJEDA GONZALEZ B, ET AL. Combination of tamoxifen and adriamycin in the treatment of advanced breast cancer. Oncology 80:111-113, 1977.
7. COLE M, JONES C, and TODD I. The treatment of advanced carcinoma of the breast with the anti-oestrogenic agent tamoxifen (ICI 46,474)—a series of 96 patients. Adv Antimicrob Antineoplas Chemother 2:529-531, 1972.
8. HENDRICK A, and SUBRAMANIAN V. Tamoxifen and thromboembolism. JAMA 243:514-515, 1980.
9. MOURIDSEN H, PALSHOF T, PATTERSON J, ET AL. Tamoxifen in advanced breast cancer (review). Cancer Treat Rev 5:131-141, 1978.
10. MOURIDSEN H, PALSHOF T, and ROSE C. Therapeutic effect of tamoxifen alone versus tamoxifen in combination with gestagen and oestrogen in advanced breast cancer. Recent Results Cancer Res 71:169-171, 1980.
11. NEVASAARI K, HEIKKINEN M, and TASKINEN P. Tamoxifen and thrombosis. Lancet 2:946-947, 1978.
12. ROESDAHL K. Tamoxifen treatment of advanced breast carcinoma. Acta Radiol Oncol 18:203-208, 1979.
13. VERONESI A, FRUSTACI S, TIRELLI U, ET AL. Tamoxifen therapy in postmenopausal advanced breast cancer: efficacy at the primary tumor site in 46 evaluable patients. Tumori 67:235-238, 1981.
14. WESTERBERG H, NORDENSKJOLD B, DE SCHRYVER A, ET AL. Anti-oestrogen therapy of advanced mammary carcinoma. Acta Radiol Ther Phys Biol 15:513-518, 1976.

15. TROUSSEAU A. Phlegmatia alba dolens. *In* Clinique Medicale de l'Hotel-Dieu de Paris, 2nd ed. Paris, J-B Bailliere, 1865, vol 3, pp 654-712.

16. ENCK R, CUNNINGHAM V, and RIOX C. Tamoxifen treatment of metastatic breast cancer and anti-thrombin III levels. Proc Am Assoc Cancer Res and ASCO 22:354, 1981.

Combination Chemotherapy With Cisplatin and Bleomycin in Advanced Cervical Cancer[1]

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Seventeen patients with advanced and recurrent squamous cell cervical cancer were treated with a combination of cisplatin and bleomycin. One complete and nine partial responses were observed (53%). The median duration of response was 8 months and the median survival was 12.7 months (5 months for nonresponders). Response was documented, particularly in patients with nodal disease and lung metastases. No life-threatening toxic effects were reported. This combination appears to be as effective as other combinations, has low toxicity, is well-tolerated, and merits further study. [Cancer Treat Rep 68:891–893, 1984]

In developed countries, cervical cancer is often diagnosed in its early curable stages, whereas in third world countries, patients often present initially with stage IV disease (11% at Groote Schuur Hospital). Treatment modalities remain limited, with palliative radiotherapy being used most frequently and chemotherapy being reserved for patients failing regional therapies. Single-agent chemotherapy has proved disappointing, with cisplatin (DDP) producing the best results [14% complete and 36% partial response rates (1, 2)]. Combination chemotherapy with a variety of agents, including DDP, has resulted in an overall response rate of 49% (3).

MATERIALS AND METHODS

The following eligibility criteria were required for admission to the study:

(*a*) Initial presentation with advanced (stage IV, FIGO criteria) cervical cancer. Eleven patients were stage IVA and six were stage IVB.

(*b*) Recurrent cervical carcinoma after a full course of radiation therapy.

(*c*) Histological documentation as squamous cell tumors.

(*d*) Evaluation of measurable parameters (in particular, tumor size) by clinical examination.

(*e*) X-ray studies, including lymphangiography or computerized tomographic studies.

(*f*) Absence of significant renal function impairment and pulmonary insufficiency.

(*g*) A minimum life expectancy of 6 weeks.

(*h*) Adequate hepatic and bone marrow functions.

The nodal recurrences were documented by a combination of methods, which included fine needle biopsy and biopsy at laparotomy, as well as lymphangiography.

Each treatment cycle consisted of 20 mg/m^2 iv of DDP on Days 1–5 and 30 mg/m^2 iv of bleomycin (BLM) on Day 2 of the initial cycle and then weekly for 12 weeks. A total dose of 360 mg/m^2 of BLM was never exceeded. Patients were evaluated at monthly intervals and after completion of four courses lasting 12 weeks, remission status was assessed critically. Patients with complete remission received an additional two courses of DDP at 3-week intervals; those achieving a partial remission received an additional course of DDP. If this last course induced complete remission, a single further course of DDP was given. The maximum number of courses administered did not exceed six. Hematologic and pulmonary functions were monitored in the usual way, the latter after three courses of treatment or earlier if indicated by the development of respiratory symptoms. DDP was administered in the now well-described manner utilizing prehydration, maintenance hydration, furosemide, and mannitol.

Complete response was defined as no evidence of disease detected by the diagnostic methods detailed above.

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Partial response was defined as a > 50% reduction in tumor size using the above methods.

RESULTS

Seventeen patients with advanced or recurrent cervical cancer were available for evaluation (table 1). Eight patients had previously received a full course of radical radiotherapy and nine were treated initially with the above cytotoxic regimen. The group of eight patients had presented a variable time period before, two with stage IIB, two with stage IIIA, and four with stage IIIB tumors. In the group of nine patients, seven presented with stage IVA and two presented with stage IVB disease.

Only one complete response was observed, in a patient with a single pulmonary metastasis proven by fine needle aspiration cytology, and this was excised after chemotherapy. Histology revealed no viable tumor. Nine partial responses were achieved, eight of these patients having completed chemotherapy. Five patients with a partial response had received previous radiotherapy, while four had had no previous treatment. In three patients, the partial response was observed in the pelvis only; in another three patients, there was documented nodal response only. In two patients, response was in both pelvis and nodes and, in one patient, response was noted in lung metastases. In seven patients, there was no response but, of these patients, only two completed the course of chemotherapy. Of the remaining five patients, treatment was abandoned in three after three courses of DDP because of lack of response and two refused treatment after two and four courses of DDP.

The median duration of response was 8 months (range, 5-15). Twelve patients have died. The median survival time for partial responders was 12.7 months (range, 7-20) versus 5.0 months for nonresponders. Five patients are still alive, four having achieved partial responses and one having achieved a complete response.

Toxic effects noted in all patients were alopecia and nausea and vomiting. Impairment of renal function occurred in one patient with rapid recovery; pulmonary toxicity did not occur. Both drugs contributed to the alopecia, with BLM being the major offender. Hematological toxicity was infrequent and usually mild; only one patient had a wbc count nadir < 2000/mm^3. No patient had a platelet count nadir < 100,000/mm^3, but hemoglobin values < 10 g/day were noted in six patients.

DISCUSSION

For many reasons, chemotherapy has been neglected in the treatment of recurrent, advanced, or metastatic squamous cell carcinoma. In particular, the problems associated with the administration of these agents and the problems associated with previous irradiation and obstructive uropathy, infection, and general debility have militated against their use. Miyamoto's (4) complete response rate of 80% with a sequential combination of BLM and mitomycin has not been substantiated by other reports (5-7).

DDP combination regimens have produced an overall

TABLE 1.—Patient characteristics and responses*

Patient No.	Disease site	Previous treatment	Completed chemotherapy	Response site	Response	Duration of response (mos)	Progression site	Survival (mos)
1	CP, IN	RT	No	Nodal	PR	5	Pelvis	7
2	CP, IN	None	Yes		NR	—	—	6
3	CP, IL	Surgery and RT	Yes	Pelvis	PR	7	Pelvis	10
4	CP, IN	None	Yes	Pelvis; nodal	PR	6	Pelvis	13
5	CP, B	None	No	—	NR	—	—	4
6	CP	None	Yes	Pelvis	PR	9	Pelvis	15
7	CP, IN	None	Yes	Nodal	PR	5	Pelvis	7
8	CP, R	None	No	—	NR	—	—	3
9	CP, B and R	None	Yes	—	NR	—	—	4
10	IN	RT	Yes	Nodal	PR	15	Nodal	20
11	CP	RT	Yes	Pelvis	PR	7	Pelvis	9
12	L	RT	Yes	Lung	CR	30	None	30
13	CP	RT	No	Pelvis	NR	—	—	4
14	CP, IL	RT	Yes	Pelvis; nodal	PR	9	Pelvis	18
15	CP	RT, surgery	No	—	NR	—	—	10
16	L	None	Yes	Lung	PR	9	Lung	16
17	CP, B	None	No	—	NR	—	—	4

* CP = central pelvis; IN = inguinal nodes; IL = iliac nodes (external/internal); B = bladder; R = rectum; L = lung; RT = radiation therapy; PR = partial response > 50%; NR = no response; CR = complete response.

response rate of 49% (3) and the combination of DDP and doxorubicin in a small series (five patients) was reported as producing a partial response rate of 20% (8). Studies incorporating BLM, DDP, vincristine, and mitomycin had a reported complete response rate of 24% and partial response rate of 54% (9,10).

The response rate in this series was 53%, with one complete response with the patient still alive and with no detectable disease after 36 months. The median duration of response was 8 months and the median survival time of partial responders was 12.7 months, compared to only 5 months in nonresponders. Predictably, the response appears to occur more frequently in patients with nodal disease and lung metastases outside the field of previous radiotherapy.

Although with present cytotoxic regimens advanced cervical cancer is rarely curable, progress with palliation has been documented and this study has been sufficiently encouraging to merit continuing investigation.

REFERENCES

1. WASSERMAN TH, and CARTER SK. The integration of chemotherapy into combined modality treatment of solid tumours: VII. Cervical cancer. Cancer Treat Rev 4:25-46, 1977.
2. THIGPEN JP, SHINGLETON M, HOMESLEY H, ET AL. Cisplatin in the treatment of advanced or recurrent cervix and uterine cancer. *In* Cisplatin: Current Status and New Developments (Prestayako AW, Crooke ST, and Carter SK, eds). New York, Academic Press, 1980, pp 411-421.
3. MUSCATO MS, PERRY MC, and YARBRO JW. Chemotherapy of cervical carcinoma. Semin Oncol 9:373-387, 1982.
4. MIYAMOTO T, JAKABE Y, WATANABE M, ET AL. Effectiveness of a sequential combination of bleomycin and mitomycin C on advanced cervical cancer. Cancer 41:403-411, 1978.
5. GREENBERG BR, HANNIGAN J, GERRETSON L, ET AL. Sequential combination of bleomycin and mitomycin in advanced cervical cancer — an American experience: A Northern California Oncology Group study. Cancer Treat Rep 66:163-165, 1982.
6. PETRILLI ES, CASTALDO TW, BALLON SC, ET AL. Bleomycin, mitomycin C therapy for advanced squamous carcinoma of the cervix. Gynecol Oncol 9:292-297, 1980.
7. BOICE CR, FREEDMAN RS, HERSON J, ET AL. Bleomycin and mitomycin C in recurrent squamous uterine cervical carcinoma. Cancer 49:2242-2245, 1982.
8. VOGL S, OHNUMA T, PERLOFF M, ET AL. Combination chemotherapy with adriamycin and cis-diamminedichloroplatinum in patients with neoplastic diseases. Cancer 38:21-26, 1976.
9. VOGL SE, MOUKHTAR M, CALANOG A, ET AL. Chemotherapy for advanced cervical cancer with bleomycin, vincristine, mitomycin C, and *cis*-diamminedichloroplatin(II) (BOMP). Cancer Treat Rep 64:1005-1007, 1980.
10. MOUKHTAR M, VOGL SE, and KAPLAN BH. Cisplatin based combination chemotherapy of advanced cancer of the uterine cervix. Proc Am Assoc Cancer Res and ASCO 20:385, 1979.

Long Persistence of Doxorubicin in Human Skin After Extravasation[1]

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Extravasation of doxorubicin (DXR) from a dorsal hand vein is described. At 28 days following this event, high dermal concentrations of DXR were still present, not only at the injection site (1.7×10^{-6} M · g^{-1}) but also at a distance of 5 cm (0.4×10^{-6} M · g^{-1}). This long persistence of DXR in the skin necessitates ample excision of the skin following severe extravasation of the drug. [Cancer Treat Rep 68:895–896, 1984]

The anthracycline antitumor antibiotics are of established efficacy in the treatment of human cancer. Of these, doxorubicin (DXR) is a major constituent of many chemotherapeutic regimens (1). Generally, the drug is administered iv. Extravasation of DXR has been reported (2-7) and may lead to severe complications. The local reaction often includes phlebitis and cellulitis and may lead to tissue necrosis. The duration of symptoms may be up to 4 months.

In this report, we describe a case of DXR extravasation where we were able to perform measurements of DXR tissue concentrations at 1 month following administration.

CASE REPORT

A 26-year-old white female with stage III-B Hodgkin's lymphoma started receiving DXR after previous treatment with MOPP (mechlorethamine, vincristine, prednisone, and procarbazine), cyclophosphamide, lomustine, mitomycin, and cytarabine. DXR was administered in an ABVD (DXR, bleomycin, vinblastine, and dacarbazine) schedule at a dose of 25 mg. It was diluted in saline to a final concentration of 5 mg/ml and administered as a rapid bolus injection through a 21-gauge butterfly needle into a vein on the dorsum of the right hand at a rate of 2 ml/minute. After administration of DXR, subcutaneous swelling around the injection site and redness of the skin were noted. Corticosteroids (hydrocortisone, 200 mg) were immediately administered locally. One week later, edema and redness of the dorsum of the hand developed, which gradually progressed to ulcerations with central necrosis. After 1 month, there was a 6 × 8-cm ulcer; necrosis expanded to the subcutis and was surrounded by reddened skin. The necrotic area was excised at Day 28 after extravasation and a split-skin graft was placed over the wound. Postoperatively, necrosis again developed, necessitating plastic surgery. The patient presently has loss of active extension of three fingers. She has been in complete remission for 4 months. The excised skin was stored at -20°C until determination of DXR levels. Histologic examination of the ulcer was performed at the same time.

RESULTS

Determinations of DXR concentrations were performed on biopsies, mechanically homogenized, and taken from the central and peripheral parts of the excised skin ulcer using high-pressure liquid chromatography according to the method described by Baurain et al (8) for tissue extraction and analysis of anthracyclines. With this method, the calculated recovery of DXR is approximately 90%.

At 28 days after extravasation, surprisingly high concentrations of DXR were found in the excised skin. In the central part of the ulcer, close to the injection site, a concentration of 750 ng/g of wet tissue was found (1.7×10^{-6} M/g of wet weight). In the peripheral part of a 5-cm distance from the injection site, a concentration was determined of 180 ng/g of tissue (0.4×10^{-6} M/g of wet weight). No metabolites of DXR were present in these samples. Histopathologic analysis performed on the excised skin showed complete epidermal destruction with epidermal and subcutaneous necrosis and reactive hyperplasia and fibrosis. This is a typical picture of DXR-induced necrosis.

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DISCUSSION

Extravasation of DXR is a severe complication, especially when it occurs on the dorsum of the hand. It usually leads to ulceration, necrosis, and reduced vitality and hand function. As has been indicated by Bowers and Lynch (6) and by Rudolph et al (4), DXR ulcers of the hand are not easy to treat and they often make repeated skin grafting necessary. These investigators suggested local recycling of DXR, possibly because as some affected cells are killed, the drug dissociates and binds to surrounding viable cells, eventually causing their death. Zweig et al (9) postulated that DXR remains present as a DXR-RNA complex in the wound.

This study demonstrates that such an event may indeed take place. In this patient, very long persistence of extravasated DXR in skin is demonstrated. Moreover, although the highest concentration was found at the injection site, a considerable concentration was also present at a distance of 5 cm. For comparison, the concentration found at the injection site at 28 days following extravasation was the same as the plasma peak concentration after iv administration of DXR. It also exceeds the skin concentration found at 2 hours after iv administration (10). Thus, it must be concluded that the extravasated DXR remains at the local site for a long period. These results are comparable with those of Garnick et al (11), who also found long persistence of DXR in the human skin following extravasation. In their patients, the amount of extravasated DXR did not precisely correlate with the dermal concentration found at 1 week to 5½ months following extravasation. In our patient, we were not able to exactly quantify the amount of extravasated DXR. However, the dermal concentration at 1 month was considerably larger than that found previously (11). It has been reported that DXR skin toxicity after extravasation can be treated by local administration of corticosteroids (12), beta-adrenergic compounds (13), or sodium bicarbonate (14). However, none of these methods seems to be really effective. In view of the results presented here, it may be postulated that the long persistence of DXR in the ulcer may contribute to the vast necrosis and poor healing. Therefore, pharmacologic antidotes cannot be expected to completely prevent the effects of DXR extravasation. Instead, early skin excision followed by split-skin grafting appears to be a logical and effective treatment, as suggested by Laughlin et al (7).

REFERENCES

1. CARTER SK. The clinical evaluation of analogs III: anthracyclines. Cancer Chemother Pharmacol 4:5-10, 1980.
2. WANG JJ, CORTES E, SINKS LF, ET AL. Therapeutic effect and toxicity of adriamycin in patients with neoplastic disease. Cancer 28:837-843, 1971.
3. TAN C, ETCUBANAS E, WOLLNER N, ET AL. Adriamycin, an antitumor antibiotic in the treatment of neoplastic diseases. Cancer 32:9-17, 1973.
4. RUDOLPH R, STEIN RS, and PATILLO RA. Skin ulcers due to adriamycin. Cancer 38:1087-1094, 1976.
5. REILLY JJ, NEIFELD JP, and ROSENBERG SA. Clinical course and management of accidental adriamycin extravasation. Cancer 40:2053-2056, 1977.
6. BOWERS DG, and LYNCH JB. Adriamycin extravasation. Plast Reconstr Surg 61:86-92, 1978.
7. LAUGHLIN RA, LANDEEN JM, and HABAL MB. The management of inadvertent subcutaneous adriamycin infiltration. JAMA 137: 408-412, 1979.
8. BAURAIN R, DEPREZ-DE CAMPENEERE D, and TROUET A. Determination of daunorubicin, doxorubicin and their fluorescent metabolites by high pressure liquid chromatography. Cancer Chemother Pharmacol 2:11, 1979.
9. ZWEIG JI, KABAKOW B, WALLACH RC, ET AL. Rational effective medical treatment of skin ulcers due to adriamycin. Cancer Treat Rep 63:2101-2103, 1979.
10. LEE YT, CHAN KK, HARRIS PA, ET AL. Distribution of adriamycin in cancer patients. Cancer 45:2231-2239, 1979.
11. GARNICK M, ISRAEL M, KHETARPAL V, ET AL. Persistence of anthracycline levels following dermal and subcutaneous adriamycin extravasation. Proc Am Assoc Cancer Res and ASCO 22:173, 1981.
12. DORR RT, ALBERTS DS, and CHEN HSG. The limited role of corticosteroids in ameliorating experimental doxorubicin skin toxicity in the mouse. Cancer Chemother Pharmacol 5:17-20, 1980.
13. DORR RT, and ALBERTS DS. Pharmacologic antidotes to experimental doxorubicin skin toxicity: a suggested role for beta-adrenergic compounds. Cancer Treat Rep 65:1001-1006, 1981.
14. IGNOFFO RJ, and FRIEDMAN MA. Therapy of local toxicities caused by extravasation of cancer chemotherapeutic drugs. Cancer Treat Rev 7:17-27, 1980.

Etoposide Infusions for Treatment of Metastatic Lung Cancer[1]

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Etoposide was given by three weekly 24-hour infusions (600 mg/m²) to 35 patients with extensive-stage lung cancer. Only two partial responses were achieved; both were of short duration. Pharmacokinetic data suggested that low peak serum concentrations of etoposide were obtained, and it is suggested that if this method of administration is used, higher doses of etoposide be employed. [Cancer Treat Rep 68:897-899, 1984]

Etoposide has been shown to be one of the more active single agents used in the treatment of small cell lung cancer (SCLC) (1,2), with up to 50% of previously untreated patients achieving a response. Results from phase II studies in non-small cell lung cancer (NSCLC) have been less encouraging. Eagan et al (3) conducted a trial in which 44 patients with evaluable NSCLC were treated with 140 mg/m² of etoposide on Days 1, 3, and 5 every 4-5 weeks. An 18% overall tumor regression rate was achieved. Several other studies have produced varying results but all have employed different dosages and schedules of administration of the drug, making comparison difficult. No phase II studies using etoposide given by infusion have been reported.

Most studies employ etoposide doses of 100-120 mg/m² given daily for 3-5 successive days, with the total dose for each treatment being 400-700 mg/m². Experiments in animal tumor models have shown that smaller, more frequent doses of epipodophyllotoxins resulted in greater increases in life span than large, weekly doses (4-6). Since extending the duration of drug administration may correlate with improved response, single-agent etoposide was given by 24-hour infusions at a dose of 600 mg/m² to 35 patients with extensive SCLC and NSCLC.

MATERIALS AND METHODS

All patients had a biopsy-proven diagnosis of lung cancer and were found, on investigation, to have disease which extended beyond one hemithorax, ipsilateral lymph nodes, or bone marrow (ie, not clinically "limited"). Four patients had previously received chemotherapy and 13 had received radiotherapy. All patients had evidence of progressive tumor at the time of entry in the study. Pretreatment investigations included bone marrow examination, chest x-rays, and routine biochemistry including liver function tests (when these were abnormal, isotope scans were performed). Fourteen patients had SCLC and 21 had NSCLC.

Etoposide was given at a dose of 600 mg/m² by 24-hour infusion repeated every 3 weeks, at least 4 weeks after any radiotherapy. The total drug dose was divided into four or six equal aliquots and was dissolved in normal saline at a concentration not exceeding 0.4 mg/ml; the stability at this concentration was 6 hours (7). Each aliquot was freshly constituted immediately prior to administration and was given over 4-6 hours. All patients were seen 10 days after treatment and assessed for hematological and other toxic effects. Evaluable disease and performance status (as scored on the Karnofsky scale) were recorded before each administration of etoposide. Treatment was discontinued if there was evidence of disease progression and was delayed by 1 week if hematological toxicity (wbc count $< 3000/\mu l$; platelet count $< 100{,}000/\mu l$) occurred. A maximum of six courses of etoposide were given to any one patient.

Response was assessed by standard criteria. Complete remission was defined as the disappearance of all known disease; partial remission was defined as a $\geq 50\%$ decrease in the sum of the products of perpendicular diameters of measurable lesions, lasting > 4 weeks; disease progression was defined as the appearance of new lesions or an increase of $> 25\%$ in the sum

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of the products of perpendicular diameters of measurable lesions, lasting > 4 weeks; and stable disease was defined as neither a 25% increase nor a 50% decrease in the size of measurable lesions.

Serial serum concentrations of etoposide were determined from 0 to 24 hours in seven patients, using an unambiguous fluorescent high-performance liquid chromatography assay developed in our laboratory. Separation and quantitation were achieved with the use of a Wate's Associates Model 440 liquid chromatograph equipped with a μBondapak phenyl reverse-phase column. The mobile phase was acetonitrile:water (50:50), and detection was by means of a Schoeffel FS970 fluorescence detector with an excitation wavelength of 240 nm and an emission filter of 380 nm. Duplicate aliquots of 10–20 μl were applied directly to the column. The lower limit of detection was 1 μmol, and in all instances calibration curves constructed for quantitation from known standards were linear from 1 μmol to 1 mmol.

RESULTS

A total of 113 courses of etoposide were given to the patient group. A median of three courses were given to each patient; five patients received six courses, and five received only one. All patients were evaluable for toxicity and response.

Toxicity

The drug was generally well-tolerated. Nausea was uncommon and vomiting did not occur. Two patients receiving more than three courses had partial alopecia. Hematological toxicity was graded according to the scale of Miller et al (8) and the maximum count depression for all courses was recorded for each patient. The hemoglobin fell to < 10 g/dl in only two patients. The wbc count was never depressed in 19 patients and fell to between 2.0 and 2.9 × 10^3/μl in ten and to between 1.0 and 1.9 × 10^3/μl in four. Neutropenia of < 1000 cells/μl was recorded in two patients. Platelet count toxicity was not seen in 80% of the patients; six patients had grades 1, 2, and 3 (two patients at each toxicity level). No treatment delays occurred as a result of hematological toxicity. Twenty-five patients never experienced an infection during treatment. Six patients were given prescriptions for oral antibiotics at some stage, two were admitted to the hospital with life-threatening infections which required parenteral antibiotics, and one died from septicemia.

Response

For 22 patients, an improvement in the performance status compared to the pretreatment level was recorded during therapy. Only improvements of ⩾ 4 weeks' duration are included.

When response in terms of tumor bulk regression was evaluated, no complete remissions and only three partial remissions were achieved. The durations of partial remissions were 6 weeks (two patients) and 9 weeks (one). Two of the responses were in undifferentiated carcinoma and one was in oat cell carcinoma.

Survival

The median survival of the total patient group was 9 months from time of diagnosis and 3 months from start of treatment. There was no significant difference in survival between the different histological subgroups. Fourteen patients were alive at the time of analysis and two have been followed for 9 months.

Pharmacokinetics

The results of pharmacokinetic studies of serum etoposide levels showed that a biphasic decay curve was identified with an α-half-life of 20 ± 2.5 minutes and a β-half-life of 6.1 ± 0.5 hours. These data would fit a closed two-compartment model using the equation

$$C^t = 277^{-2.4t} + 112^{-0.11t},$$

giving an area under the curve of 1009 ± 129 μmols . L/hour and a drug clearance of 33 ± 5/ml/minute. A mean peak serum concentration of 390 μmols was obtained.

CONCLUSIONS

The disappointing results for the 35 patients who were treated with infusions of etoposide for metastatic lung cancer confirm that this dosage or schedule is ineffective for all histological subtypes. Pharmacokinetic data which were obtained during this trial suggested that ineffective drug levels were achieved.

Fortunately, toxicity was minimal and inpatient stay rarely required > 1 night for each treatment.

Further use of this expensive agent requires careful evaluation of the most appropriate dosages and scheduling. Treatment using etoposide given by infusion may prove to be more effective than giving it by intermittent bolus, but higher doses than were used in this trial will need to be given.

REFERENCES

1. EAGAN RT, CARR DT, FRYTAK S, ET AL. VP-16-213 versus polychemotherapy in patients with advanced small cell lung cancer. Cancer Treat Rep 60:949–951, 1976.

2. TUCKER RD, FERGUSON A, VAN WYCK C, ET AL. Chemotherapy of small cell lung cancer with VP16 213. Cancer 41:1710-1714, 1978.
3. EAGAN RT, INGLE JN, CREAGAN ET, ET AL. VP-16-213 chemotherapy for advanced squamous cell carcinoma and adenocarcinoma of the lung. Cancer Treat Rep 62:843-844, 1978.
4. ROZENCWEIG M, VON HOFF D, HENNEY JE, ET AL. VM26 and VP16 213: a comparative analysis. Cancer 40:334-342, 1977.
5. DOMBERNOWSKY P, and NISSEN NI. Schedule dependency of the antileukaemic activity of the podophyllotoxin derivative VP16 213 (NSC 141540) in L1210 leukaemia. Arch Pathol Microbiol Scand 81:715-724, 1973.
6. VENDITTI JR. Treatment schedule dependency of experimentally active antileukemic (L1210) drugs. Cancer Chemother Rep Part 3, vol 2(1):35-59, 1971.
7. BRISTOL LABORATORIES. Product Development Research Reports, 1980.
8. MILLER AB, HOOGSTRATEN B, STAQUET M, ET AL. Reporting results of cancer treatment. Cancer 47:207-214, 1981.

Sinus Histiocytosis With Massive Lymphadenopathy: Response to Cyclophosphamide Therapy[1]

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A patient with sinus histiocytosis with massive lymphadenopathy is described. After attempts to control lymph node size and constitutional symptoms with corticosteroids were unsuccessful with radiation therapy and nonsteroidal anti-inflammatory drugs, the patient was treated with two courses of low doses of oral cyclophosphamide therapy. He remains in complete remission 15 months after therapy was discontinued. [Cancer Treat Rep 68:901–902, 1984]

Sinus histiocytosis with massive lymphadenopathy (SHML) was described by Rosai and Dorfman (1) as a clinicopathological entity consisting of cervical lymphadenopathy, which on biopsy shows marked dilatation of the lymph node sinuses containing histiocytes. Subsequently, patients with involvement of virtually all lymph node-bearing regions as well as extranodal sites have been described (2). SHML is considered to be a benign disorder, although treatment with corticosteroids, irradiation and, more recently, chemotherapy has been used to control symptoms (3).

Here we report a patient with SHML with severe constitutional symptoms and mechanical complications secondary to the massive cervical adenopathy unresponsive to corticosteroids or radiation therapy, who achieved a complete remission after oral cyclophosphamide chemotherapy.

CASE REPORT

A 25-year-old previously healthy white male presented in May 1980 with a 2-month history of low-grade fevers and tender left submandibular adenopathy. He was treated with oral antibiotics for 2 weeks with no response. The patient had a lymph node biopsy that was thought to be consistent with Hodgkin's disease. He was referred to the University of Chicago and was admitted on June 10, 1980 for evaluation.

The patient reported 2 months of daily fevers to 100°F, drenching night sweats, and a 10-pound weight loss over 4 weeks. Physical examination was significant for the following: a 7 × 4-cm left submandibular lymph node (LN), a 4 × 5-cm left anterior cervical LN, a 3 × 5-cm right submandibular LN, and a 1-cm right posterior cervical LN. There was mild lymphoid hyperplasia of Waldeyer's ring. There were no palpable abdominal masses or organomegaly. The remainder of the physical examination was normal.

Laboratory values were as follows: wbc count, 9.7 × $10^3/mm^3$; hemoglobin, 14.7 g/100 ml; hematocrit, 44%; platelet count, $382,000/mm^3$; and sedimentation rate (Westergren), 38 mm/hour. Screening chemistries of liver and renal functions were all within normal range. Rheumatoid factor, antinuclear antibody, and Coombs' test were negative. Serologies for cytomegalovirus, Epstein-Barr virus, and toxoplasma were negative. Intermediate-strength purified protein derivative was negative with a positive streptokinase-streptodornase control. Serum protein electrophoresis showed mild polyclonal expansion of IgA and IgG. Chest x-ray revealed left hilar adenopathy and a lytic lesion in the third right rib. Computerized tomography of the abdomen showed no evidence of retroperitoneal adenopathy.

Review of the pathological material obtained from the lymph node biopsy was felt to be diagnostic of SHML. A bone marrow biopsy showed an increase in marrow histiocytes but was otherwise nondiagnostic.

The patient was discharged on the fourth hospital day with lymph node tenderness. He was given aspirin therapy for fevers; this therapy was not effective, and in July 1980 he started receiving prednisone (60 mg/day), with disappearance of his fevers but no change in lymphade-

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nopathy. After 6 weeks, the steroids were tapered and discontinued with prompt re-emergence of his constitutional symptoms. In December 1980, he received 2000 rads to the anterior and posterior opposing poles encompassing the entire neck, with a midline block from a 4-MeV linear accelerator. He achieved a 40% reduction in LN size lasting 4 weeks, after which his adenopathy returned to pretreatment size. Constitutional symptoms persisted. A therapeutic trial of indomethacin was ineffective.

Through 1981, the patient's submandibular and cervical lymphadenopathy gradually enlarged, causing severe limitation in neck range of motion. Significant right paratracheal adenopathy became apparent on x-ray. On September 2, 1981, after obtaining informed consent, the patient started receiving cyclophosphamide (50 mg/day orally). Over 4 weeks, there was a 70% reduction in the lymph node size and complete abrogation of all constitutional symptoms. At the completion of 6 weeks of cyclophosphamide therapy, there was no palpable adenopathy and treatment was discontinued.

In March 1982, there was re-emergence of the cervical adenopathy and cyclophosphamide therapy was reinstituted, initially at 150 mg/day for 14 days, then at 50 mg/day orally, for a total of 12 weeks. There was no significant change in blood cell counts throughout therapy. No other toxic effects were reported.

The patient has been asymptomatic, in continuous complete remission, since the discontinuation of therapy.

DISCUSSION

SHML is considered to be a benign disorder. It may, however, behave in an aggressive fashion associated with significant morbidity and, rarely, mortality. Patients with massive skin tumors, orbital and nasal involvement, bone involvement, and paraplegia secondary to spinal epidural disease have been reported previously (1-4). Our patient had significant decrease in neck range of motion, severe constitutional symptoms, and marked paratracheal lymphadenopathy necessitating palliative therapy.

Although the exact etiology is not known, Lampert and Lennert (5) suggest that SHML may represent a disordered response to antigen stimulation (ie, *Klebsiella* antigen or Epstein-Barr virus) or a manifestation of impaired cell-mediated immunity. Morphologically, the disease is most like the disorders of the mononuclear phagocyte lineage, the histiocytoses, with striking dilatation of the lymph node sinuses laden with foamy macrophages and the presence of cytophagocytosis. There is in vitro increase in mononuclear cell phagocytosis as well.

In most of the patients described, SHML has a variable course with spontaneous remission occurring on no therapy over 3-6 months. Corticosteroids have been used in the treatment of SHML with inconsistent results. Lampert and Lennert (5) reported on two patients who experienced significant long-term remission after prednisone therapy. In our patient, however, corticosteroid therapy was most effective in abrogating constitutional symptoms and had little or only transient effect on lymph node size.

More recently, vinblastine-loaded platelets from a patient with idiopathic thrombocytopenic purpura have been used to treat SHML (6). The patient described had dramatic, although transient, regression of massive skin involvement and lymphadenopathy. The rationale for using this form of therapy was to selectively attack the abnormal cell population active in phagocytosis with relative sparing of other cell lines. Although effective, the response described was of short duration.

Cyclophosphamide has been used successfully in the treatment of malignancies of the monocyte-macrophage system (7). Our patient achieved a complete remission after each course of low-dose cyclophosphamide and remains in continuous complete remission 15 months after all therapy was discontinued. The total dose of alkylating agent was approximately 8 g given over a 5-month period. The use of alkylating agents can be associated with significant short- and long-term toxicity, specifically, the development of secondary malignancy. For this reason, we do not advocate the routine use of cyclophosphamide therapy in SHML where spontaneous remissions are not unusual. We feel, however, that in circumstances where corticosteroids and radiation therapy are not effective and significant morbidity is present, it is a reasonable form of therapy that can produce a rapid and durable response.

REFERENCES

1. ROSAI J, and DORFMAN RF. Sinus histiocytosis with massive lymphadenopathy. Arch Pathol 87:63-70, 1969.
2. ROSAI J, and DORFMAN RF. Sinus histiocytosis with massive lymphadenopathy—a pseudolymphomatous benign disorder. Analysis of 34 cases. Cancer 30:1174-1188, 1972.
3. HAAS RJ, HELMIG MSE, and PRECHTEL K. Sinus histiocytosis with massive lymphadenopathy and paraparesis: remission with chemotherapy. Cancer 42:77-80,1978.
4. KESSLER E, SRUCIJES C, TOLEDO E, ET AL. Sinus histiocytosis with massive lymphadenopathy and spinal epidural and bone involvement. Cancer 38:1614-1618, 1976.
5. LAMPERT F, and LENNERT K. Sinus histiocytosis and massive lymphadenopathy. Cancer 37:783-789, 1976.
6. PENNEYS NS, AHN YS, MCKINNEY EC, ET AL. Sinus histiocytosis with massive lymphadenopathy. A case with unusual skin involvement and a therapeutic response to vinblastine loaded platelets. Cancer 49:1994-1998, 1982.
7. ALEXANDER M, and DANIELS JR. Chemotherapy of malignant histiocytosis in adults. Cancer 39:1011-1017, 1977.

Mitoxantrone in Malignant Melanoma[1,2]

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Twenty-nine patients with metastatic malignant melanoma without prior chemotherapy and 36 patients refractory to prior chemotherapy received mitoxantrone (5 mg/m²/week). No patient achieved a partial response. The dose-limiting toxic effect was granulocytopenia. Mitoxantrone is ineffective in malignant melanoma. [Cancer Treat Rep 68:903–905, 1984]

Mitoxantrone has been demonstrated in vitro to produce antitumor effects similar to those of doxorubicin, and it may produce less cardiotoxicity than doxorubicin (1). To try to develop new agents with significant antitumor activity and decreased toxicity, mitoxantrone has been studied in clinical pharmacological studies (2) as well as in phase I toxicologic studies (3–5). Based on the various schedules which have been found to be tolerated, phase II studies have been proposed and initiated (6–9).

Since it has been recognized that drug activity in in vitro soft agar colony-forming assays has correlated well with the activity of such drugs utilized clinically in predominantly drug-resistant patients (10), it has been proposed that the use of such assays might be valuable in selecting agents of potential clinical effectiveness for subsequent phase II trials to determine antitumor effects. It was therefore of considerable interest that the testing of mitoxantrone in such a culture system demonstrated excellent in vitro antitumor activity against specimens from patients with malignant melanoma (10). The Southeastern Cancer Study Group (SEG), therefore, elected to study mitoxantrone in a phase II trial in patients with incurable malignant melanoma; some had previously shown drug resistance and the rest had not received chemotherapy. This article summarizes the results of SEG Protocol 80 MEL 277.

METHODS

Sixty-five patients with metastatic malignant melanoma were entered in this protocol: 29 with no prior chemotherapy and 36 with prior chemotherapy. Eligibility requirements included biopsy-proven malignant melanoma incurable by standard surgical means, pretreatment performance status > 30%, wbc count > 4000/dl, platelet count > 100,000/dl, and lack of hyperbilirubinemia or azotemia. Patients must have given voluntary informed consent prior to participation, and they must have had no evidence of pre-existing, uncompensated cardiac disease.

Patients were treated weekly with mitoxantrone (3). Mitoxantrone was injected iv at a dose of 5 mg/m²/week for 6 weeks. Patients who had stable disease or partial response continued to receive therapy, while patients with progressive disease were defined as treatment failures and were removed from the study. Mitoxantrone was given by 30-minute iv infusion.

If the pretreatment granulocyte count was < 1500/dl or the platelet count was < 90,000/dl for any weekly injection, the dose of mitoxantrone was decreased by 25%, and treatment was delayed until the counts rose above those levels. If there was severe vomiting, the dose of mitoxantrone was reduced by 50%. If the bilirubin rose to > 1.3 mg/dl, the dose of mitoxantrone was decreased by 50%; if it rose to > 2 mg/dl, the dose was decreased by 75%; and if it rose to > 5 mg/dl, mitoxantrone treatment was discontinued.

A partial response was defined as a > 50% reduction in the sum of the products of diameters of bidimensionally measured tumors. Disease progression was defined as a > 25% increase in the sum. Stable disease was defined as neither partial response nor disease progression.

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RESULTS

Of the 65 patients entered in this study, 29 had received no prior chemotherapy (table 1). Of those patients, 22 are currently evaluable. The remaining patients are either inevaluable (two patients) or have not been in the study long enough for analysis (five). Thirty-six patients had received chemotherapy to which they had become resistant prior to entry in this mitoxantrone trial. Of those patients, 29 are currently evaluable. Three patients did not receive adequate trials of therapy, having received only one or two weekly injections of mitoxantrone, and four patients have not been in the study long enough for analysis.

Pretreatment characteristics in terms of sex, age, pretreatment performance status, and metastatic pattern are shown in table 1.

No patient in this trial had a partial antitumor response to mitoxantrone. A small number of patients both with previously untreated and with previously treated malignant melanoma had stable disease, but most patients had disease progression while receiving mitoxantrone.

The dose-limiting toxic effect in this trial was granulocytopenia. The lowest weekly granulocyte count was < 1500/dl in 67%–80% of patients. The lowest granulocyte count was < 1000/dl in 66% of previously treated patients and in 57% of previously untreated patients. Platelet counts < 90,000/dl were rarely observed, occurring in 5% of the previously untreated patients and in 10% of the previously treated patients. Severe nausea and vomiting was uncommon in this trial. An unexplained rise in creatine to > 2 mg/dl was rarely observed in this trial. Shortness of breath, also unexplained but not associated with congestive heart failure, was observed in one patient with previously treated melanoma. Tachycardia was observed in two patients, and a decrease in voltage on ECG was observed in one. No patients had congestive heart failure. One patient reported a change in mood, and one patient had unexplained involuntary tremor during this trial.

DISCUSSION

Despite the finding of excellent antitumor activity in in vitro colony-forming assays of mitoxantrone with specimens of human malignant melanoma (10), we found no clinical activity of mitoxantrone either in patients with previously untreated malignant melanoma or in patients with previously drug-resistant malignant melanoma. We conclude that this agent is unlikely to have a clinical response rate of > 10% during palliative therapy of patients with malignant melanoma. Since doxorubicin also lacks significant clinical antitumor activity against melanoma, this suggests that anthracycline and anthracene derivatives are not likely to be efficacious in this disease.

TABLE 1.—Mitoxantrone in malignant melanoma: patient characteristics and response*

	No prior chemotherapy	Resistance to prior chemotherapy
Entered	29	36
Evaluable	22†	29
Sex		
Female	6	11
Male	16	18
Median age in yrs	53.04	46.53
Pretreatment performance status		
80–100	19	21
30–70	3	8
Metastatic pattern		
Skin and/or nodes only	8	10
Visceral involvement	14	19
Tumor response		
Partial	0	0
Stable disease	5	3
Disease progression	16	26
% with toxicity (moderate or severe)		
Granulocytopenia	67	80
Thrombocytopenia	5	10
Vomiting	14	7
Azotemia (elevated BUN)	6	0

* Unless otherwise specified, values = No. of patients.

†1 previously untreated patient was evaluable for toxicity only, since tumor was not measurable.

Future trials to identify new active agents should probably stress other classes of antitumor drugs.

Furthermore, this trial indicates that caution should be used in extrapolating the results of in vitro tumor efficacy (in colony-forming asays) to expectations for high degrees of clinical efficacy in testing phase II drugs in clinical trials. Our studies of mitoxantrone in soft tissue sarcoma (to be reported separately) also substantiate this caution.

REFERENCES

1. Clinical Brochure. 1,4-Dihydroxy-5,8-bis [[2-[(2-hydroxyethyl)amino]ethyl)amino]-9,10-anthracenedione dihydrochloride (CL 232, 315). American Cyanamid Co, 1979.
2. STEWART JA, MCCORMACK JJ, and KRAKOFF IH. Clinical and clinical pharmacologic studies of mitoxantrone. Cancer Treat Rep 66:1327-1331, 1982.
3. ALBERTS DS, GRIFFITH KS, GOODMAN GE, ET AL. Phase I clinical trial of mitoxantrone: a new anthracenedione anticancer drug. Cancer Chemother Pharmacol 5:11-15, 1980.
4. VALDIVIESO M, BEDIKIAN AY, BURGESS MA, ET AL. Phase I clinical study of dihydroxyanthracenedione administered on a 5-day iv schedule. Cancer Treat Rep 65:841-844, 1981.
5. VAN ECHO DA, WHITACRE MY, AISNER J, ET AL. Phase I trial of dihydroxyanthracenedione. Cancer Treat Rep 65:831-834, 1981.
6. YAP HY, BLUMENSCHEIN GR, SCHELL FC, ET AL. Dihydroxyanthracenedione: a promising new drug in the treatment of metastatic breast cancer. Ann Intern Med 95:694-697, 1981.
7. DE JAGER R, CAPPELAERE P, EARL H, ET AL. Phase II clinical trial of mitoxantrone: 1,4-dihydroxy-5,8-bis (((2-((2-hydroxyethyl)amino) ethyl)amino)) 9,10 anthracenedione dihydrochloride in solid tumors and lymphomas. *In* Proceedings of the Third NCI-EORTC Symposium on New Drugs in Cancer Therapy, Brussels, Oct 15-17, 1981. Belgium European Organization for Research on Treatment of Cancer, 1981, p A39.
8. GAMS RA, TERZAKIS TA, and BARTOLUCCI AA. Phase II studies of mitoxantrone. *In* Proceedings of the Third NCI-EORTC Symposium on New Drugs in Cancer Therapy, Brussels, Oct 15-17, 1981. Belgium European Organization for Research on Treatment of Cancer, 1981, p A38.
9. STEPHENS RL, and CLARK G. Southwest Oncology Group (SWOG) phase II with mitoxantrone in non-hematologic malignancies. *In* Proceedings of the Third NCI-EORTC Symposium on New Drugs in Cancer Therapy, Brussels, Oct 15-17, 1981. Belgium European Organization for Research on Treatment of Cancer, 1981, p A36.
10. VON HOFF DD, COLTMAN CA, and FORSETH B. Activity of mitoxantrone in a human tumor cloning system. Cancer Res 41:1853-1855, 1981.

Pulmonary Toxicity Associated With Spirogermanium[1]

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Spirogermanium is a new antitumor agent that was synthesized by Rice et al (1) in 1974. This compound is an azaspiran that substitutes the metal germanium for a carbon moiety in the heterocyclic ring. The mode of action producing cytolysis appears to be inhibition of protein synthesis with secondary depression of DNA and RNA synthesis. Early phase I-II studies have indicated that this agent is nonmyelotoxic, with responses seen in breast cancer and malignant lymphoma (2-4). The principal toxic effects in these trials have been varying degrees of neurologic dysfunction such as dizziness, vertigo, nystagmus, ataxia, and paresthesias. In a phase I study at the M. D. Anderson Hospital, we observed two patients with pulmonary toxicity that may have been related to spirogermanium (5); they are described here in greater detail.

CASE REPORTS

Patient 1

A 61-year-old woman developed abdominal pain in December 1978. A stage IIE, diffuse, large cell malignant lymphoma was found during a laparotomy. The patient was treated with cyclophosphamide, doxorubicin, vincristine, prednisone, and bleomycin (CHOP-Bleo), followed by 3000 rad to the upper two-thirds of the abdomen. Four additional courses of CHOP-Bleo were given followed by another 3000 rad to the pelvis (total bleomycin dose, 100 mg). She was asymptomatic until abdominal adenopathy was noted again on a lymphangiogram in January 1981. Ifosfamide, etoposide, and methotrexate were administered. A lymphangiogram and computerized tomographic scan of the abdomen were normal in January 1982. Abdominal adenopathy was found on an abdominal survey in April 1982. Spirogermanium was initiated by continuous infusion at a dose of 200 mg/m^2 (350 mg/day). Initially, the administration was complicated by depression and anxiety, but these were eliminated by a dose reduction to 180 mg/m^2 (270 mg/day). Over the next 3 months, the patient complained of intermittent fever, pleuritic chest pain, nonproductive cough, and dyspnea. Results of diagnostic studies (including numerous blood, urine, and sputum cultures; viral titers and cultures; chest radiographs; and lung and cardiac scans) were negative. An abdominal survey showed a reduction of abdominal lymphadenopathy. Spirogermanium was continued. Five months after beginning spirogermanium, dyspnea worsened and a chest radiograph showed a small right-sided pleural effusion and diffuse bilateral interstitial infiltrates. Arterial blood gas studies showed mild hypoxemia and mild respiratory alkalosis. Pulmonary function tests demonstrated a moderately reduced forced vital capacity (54% predicted), forced expiratory volume in 1 second (63% predicted), total lung capacity (74% predicted), and diffusing capacity (47% predicted). Similar studies performed 3 years earlier were all within the normal range. Multiple blood, urine, and sputum cultures for acid-fast bacillus, fungus, and *Legionella* were negative. Cold agglutin, cytomegalovirus, *Mycoplasma,* and *Toxoplasma* titers were also negative. Broad-spectrum antibiotics were begun without improvement in either symptoms or chest roentgenogram. A transbronchial biopsy revealed alveoli filled with foamy macrophages and focal nonspecific interstitial fibrosis. The stains for *Pneumocystis carinii,* acid-fast bacilli, fungi, and bacteria were unremarkable. Microbiological cultures of the specimen were negative. The patient refused to undergo an open-lung biopsy. Prednisone (60 mg/day) was given. Arterial blood gases showed gradual increasing hypoxemia. Three weeks later respiratory failure ensued, and mechanical ventilation was initiated. A single sputum culture 3 days later demonstrated *Mycobacterium avium*-intracellular complex resistance to rifampin, isoniazid, and ethambutol but sensitivity to amikacin. Despite intensive respiratory and chemotherapy support, arterial blood gases worsened, and the patient died; no autopsy was performed.

Patient 2

A 52-year-old man with chylous ascites was diagnosed

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as having stage IIE, diffuse, mixed lymphoma after an exploratory laparotomy revealed a large retroperitoneal tumor on the lower small bowel mesentery with multiple para-aortic and left renal lymph nodes involved with tumor. He received cyclophosphamide, vincristine, and prednisone, then 4000 rad to the entire abdomen, with additional mantle irradiation of 4000 rad. A computerized tomographic scan of the abdomen was normal in December 1981. CHOP was given until April 1982. In June 1982, a left cervical node biopsy revealed diffuse large cell lymphoma, and on physical examination a 1 × 2-cm right epitrochlear node was found. Spirogermanium (200 mg/m^2; 400 mg/day) was begun by continuous infusion for 24 days, with regression of the right epitrochlear node. After a 2-week rest, a second course of spirogermanium at a reduced dose of 100 mg/m^2 (200 mg/day) was initiated. Approximately 2 weeks later, the patient began to complain of shortness of breath, low-grade fever, and fatigue. On physical examination, he was tachypneic, and rales were present in both lung bases. The chest radiograph revealed bibasilar infiltrates, left pleural effusion, and volume loss. Lung function tests showed a restrictive ventilatory defect and moderate decrease in diffusion capacity. Transbronchial biopsy was performed. Histologic finding showed increase in interstitial fibrous connective tissue and foamy macrophages filling the alveoli. Spirogermanium was discontinued and prednisone (50 mg/day) was started. One month later the dyspnea was better and the chest radiograph showed improvement of the interstitial infiltrate. However, the restrictive lung defect and diffusing capacity were unchanged. A repeat chest radiograph 8 months after discontinuing spirogermanium shows no evidence of interstitial infiltrates. Additional lung function studies have not been performed.

DISCUSSION

Both patients had a clinical picture similar to that observed in patients with pulmonary toxicity caused by other chemotherapeutic agents (6). Cough, dyspnea, fatigue, and fever began insidiously and progressively worsened. Pulmonary physical findings showed tachypnea and bibasilar rales. Abnormalities in lung function consisted of reduced diffusing capacity, restrictive lung volumes, and hypoxemia. The chest radiograph revealed a diffuse bibasilar interstitial pneumonitis. The pathological findings consisted of alveoli filled with foamy macrophages and mild interstitial fibrosis.

Spirogermanium has not been reported to cause pulmonary toxicity in other phase I–II studies. However, the temporal relationship of clinical findings during spirogermanium treatment in these patients suggests a cause and effect relationship. The resultant lung injury seen in our patients could be the consequence of prolonged continuous infusion, since similar toxicity has not been observed with intermittent courses.

As with most reports concerning chemotherapy-induced pulmonary toxicity, our patients had been previously treated with other agents and both had received chest irradiation. What role these factors played is unclear, but prior to beginning spirogermanium both patients had essentially normal chest radiographs. One patient had normal lung function on previous testing. The reaction observed in our patients could theoretically be related to prior exposure to cyclophosphamide or bleomycin; however, pulmonary drug toxicity from cyclophosphamide typically occurs after prolonged exposure, and pulmonary toxic effects from bleomycin usually occur after several months of therapy with doses > 450 mg (6). Patient 1 received a total bleomycin dose of 100 mg. The possibility of disseminated *Mycobacterium avium*-intracellular in Patient 1 is considered unlikely, since multiple cultures of sputum, blood, urine, bone marrow, lung biopsy, and bronchial washing were negative the preceding month and this organism was recovered from a single specimen.

It has been over two decades since busulfan was initially reported to cause interstitial pneumonitis and pulmonary fibrosis. Since then, a number of other chemotherapeutic agents have been associated with pulmonary toxicity. We believe that spirogermanium should be added to the list of chemotherapeutic agents that can potentially cause pulmonary injury. In addition, since phase II studies with spirogermanium are being initiated, lung function may need to be monitored to detect any pulmonary change during treatment, particularly in those studies administering the drug by continuous infusion.

REFERENCES

1. RICE LM, WHEELER JW, and GESCHICKLER CF. Spirans XXII. Synthesis of 4,4-dialkyl-4-germacyclohexanone and 8,8-dialkyl-8-germaaza-spiro (4.5) decanes. J. Heterocyclic Chem 11:1041-1046, 1976.
2. SCHEIN PS, SLAVIK M, SMYTHE T, ET AL. Phase I clinical trial of spirogermanium. Cancer Treat Rep 64:1051-1056, 1980.
3. FALKSON G, and FALKSON HC. Phase II trial of spirogermanium for treatment of advanced breast cancer. Cancer Treat Rep 67:189-190, 1983.
4. MATTSON W, TROPÉ C, and ORBERT B. A phase I study of spirogermanium. Proc Am Assoc Cancer Res and ASCO 21:778, 1980.
5. LEGHA SS, AJANI JA, and BODEY GP. Phase I study of spirogermanium given daily. J Clin Oncol 1:331-336, 1983.
6. BATIST G, and ANDREWS JL. Pulmonary toxicity of antineoplastic drugs. JAMA 246:1149-1453, 1981.

Acute Myelogenous Leukemia Following Radiation Therapy and Chemotherapy for Osteogenic Sarcoma[1,2]

Andrew D. Jacobs* and Robert Peter Gale[3]

Patients receiving ionizing radiation therapy or cytotoxic chemotherapy are at increased risk of developing acute myelogenous leukemia. Ten cases of therapy-linked myelogenous leukemia have been reported in patients with sarcoma, and we report here the first case in a patient who received combined-modality therapy for treatment of an osteogenic sarcoma. As treatment for this disease becomes more intensive and survival improves, the incidence of leukemia following therapy for osteogenic sarcoma may increase. [Cancer Treat Rep 68:909–911, 1984]

Patients who receive ionizing radiation therapy or cytotoxic drugs, alone or in combination, have an increased risk of developing therapy-linked acute myelogenous leukemia (AML). Therapy-linked AML has been most commonly reported in patients treated for neoplastic diseases including multiple myeloma, ovarian carcinoma, Hodgkin's disease, non-Hodgkin's lymphoma, and polycythemia rubra vera. Drugs commonly implicated as causing therapy-linked AML include alkylating agents such as cyclophosphamide and chlorambucil, nitrosoureas, azathioprine, vinblastine, and procarbazine. Other drugs used to treat solid tumors and immune disorders are also reported to increase the risk of developing therapy-linked AML. These drugs include methotrexate, 5-FU, vinblastine, uracil mustard, and thiotepa. The leukemogenic effect of ionizing radiation is also well-documented. Patients with therapy-linked AML typically develop pancytopenia a median of 4–5 years after treatment of their original disease; < 10% of the cases occur within the first 2 years (1–3).

Only ten cases of therapy-linked AML have been reported in patients with sarcoma. Most have been in patients with soft tissue sarcomas such as fibrosarcoma and leiomyosarcoma. One case of therapy-linked AML following combined-modality treatment of Ewing's sarcoma has been described (4). In this report we describe the first case of therapy-linked AML following the treatment of osteogenic sarcoma.

CASE REPORT

A 28-year-old female was in good health until June 1977 when she developed paresthesias and swelling over the left knee. By November 1977, the swelling increased; a biopsy was performed and the tissue obtained was consistent with a diagnosis of osteogenic sarcoma.

Initial therapy consisted of an intra-arterial infusion of doxorubicin (60 mg/m^2) followed by radiation therapy (3.5 Gy) from a cobalt source, given in ten fractions to the left femur. In February 1978, a block excision of the residual tumor was performed followed by a cadaveric allograft replacement of the distal left femur. Microscopic review of the excised tumor showed a cortical osteochondroma with a focus of transformation to malignant periosteal sarcoma (grade 1). Because of tumor involvement of the proximal margin, further adjuvant chemotherapy was given consisting of five courses of doxorubicin (70 mg/m^2) over 2 days at monthly intervals, three doses of vincristine (1 mg/m^2) at monthly intervals, and 12 doses of methotrexate including three at high dose between February 1978 and April 1979. Total drug doses were 420 mg/m^2 of doxorubicin, 3 mg/m^2 of vincristine, and 66 g/m^2 of methotrexate.

In April 1980, the patient developed staphylococcal arthritis and osteomyelitis of the left knee. These infections persisted despite antibiotics, and an above-knee

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TABLE 1.—Therapy-linked AML following treatment of sarcoma

Investigators	Sarcoma	Age (yrs)	Sex	Treatment*		Interval to leukemia (mos)	Ref No.
				Chemotherapy	Radiation therapy		
Smithson et al	Ewing's sarcoma	16	M	DOX, V, AD, CY	5 Gy, local; 1.8 Gy, lungs	16	4
Wildhack	Myosarcoma	75	M	"Trenimon"	3.25 Gy	24	8
Warren and Ehrenreich	Neurogenic sarcoma	26	M	Not indicated	Not indicated	120	9
Hensley et al	Rhabdomyosarcoma	3	F	AD, V, CY, DOX, DTIC	6 Gy	36	10
Youness et al	Leiomyosarcoma	64	F	CY, V, DOX, DTIC, CP	—	24	11

*DOX = doxorubicin; V = vincristine; AD = dactinomycin; CY = cyclophosphamide; DTIC = dacarbazine; CP = cisplatin; and Gy = Gray.

amputation was performed. The patient recovered rapidly, receiving continuous oral cephalexin, and remained well until April 1981. A routine hemogram at this time revealed pancytopenia with hemoglobin of 12.3 g/dl, hematocrit of 35%, wbc count of 1.6×10^9/L, and platelet count of 4×10^9/L. A bone marrow biopsy specimen was hypercellular consisting of a diffuse infiltrate of immature myeloblasts and monoblasts with prominent nucleoli. The immature cells had histochemical features of AML, including reactivity with Sudan black and nonspecific esterase. Chromosome analysis revealed a normal 46, XX karyotype.

A diagnosis of AML was made and the patient was treated with an intensive course of cytarabine, 6-thioguanine, and daunorubicin. Remission was not achieved, and further attempts at induction chemotherapy included one course of vincristine, cyclophosphamide, cytarabine, and prednisone and one course of amsacrine. Treatment was unsuccessful and the patient died of resistant AML in December 1981.

DISCUSSION

This patient developed AML 3 years after the diagnosis and treatment of osteogenic sarcoma with local radiation therapy and adjuvant chemotherapy with doxorubicin, vincristine, and methotrexate. The combination of radiation therapy and chemotherapy has an additive mutagenic effect on hematopoietic stem cells. This is indicated by data from patients with Hodgkin's disease, where the risk of therapy-linked AML in patients receiving both radiation therapy and chemotherapy is five times higher than in patients receiving chemotherapy alone (2). Our patient, although she probably had therapy-linked AML, is unusual with regard to two factors. She received only localized radiation therapy and she did not receive drugs regarded as being highly leukemogenic.

There are ten reported cases of therapy-linked leukemia in patients with sarcomas (4-13). Our critical review indicates only five well-documented cases. One previously reported case was in a patient with fibromatosis who was mistakenly treated for rhabdomyosarcoma (5). Other reports have included cases of lymphosarcoma and "reticulosarcoma" (6,7). They were undoubtedly non-Hodgkin's lymphomas rather than sarcomas. Two other reports refer to patients who developed lymphocytic leukemia rather than AML following treatment of sarcoma. The five verified cases of therapy-linked AML in patients with sarcoma are reviewed in table 1.

Therapy-linked AML is a rare complication of sarcoma therapy. Recently, the treatment of sarcomas, particularly osteogenic and Ewing's sarcomas, has become more intensive, and combined-modality therapy is frequently used. As survival improves, therapy-linked AML may become a more frequent complication. This possibility should be considered in patients with osteogenic sarcoma who develop unexplained pancytopenia after receiving combined-modality therapy.

REFERENCES

1. CASCIATO DA, and SCOTT JL. Acute leukemia following cytotoxic agent therapy. Medicine 58:32-47, 1979.
2. ROSNER F, and GRUNWALD HW. Cytotoxic drugs and leukaemogenesis. Clin Haematol 9:663-681, 1980.
3. CRONKITE EP, MOLONEY W, and BOND VP. Radiation leukemogenesis: an analysis of the problem. Ann Intern Med 28:673-682, 1960.
4. SMITHSON WA, BURGERT EO, CHILDS DS, ET AL. Acute myelomonocytic leukemia after irradiation and chemotherapy for Ewing's sarcoma. Mayo Clin Proc 53:757-759, 1978.
5. GUTJAHR P, and SPRANGER J. Acute leukemia following anticancer treatment. J. Pediatr 87:1004-1005, 1975.
6. GUNZ FW, and ANGUS HB. Leukemia and cancer in the same patient. Cancer 18:145-152, 1965.
7. MULDER NH, and HOUWEN B. Behandelen en vooruitzien. Acute leukemie na behandeling van eeen audere kwaadaardige ziekte. Med T Geneest 122:385-388, 1978.
8. WILDHACK R. Monozytenleukamie mit γG-Paraproteinamie nach rontgennachbestrahlung und zytostatischer behandlung eines myosarkoms. Deutsch Med Wochenschr 92:255-257, 1967.
9. WARREN S, and EHRENREICH T. Multiple primary malignant tumors and susceptibility to cancer. Cancer Res 4:554-570, 1944.
10. HENSLEY MF, CANGIR A, CULBERT SJ, ET AL. Acute granulocytic leukemia following successful treatment of rhabdomyosarcoma. Am J Dis Child 131:1417, 1977.

11. YOUNESS E, DOSIK G, BENJAMIN RS, ET AL. Acute myelomonocytic leukemia following a chemotherapeutic regimen for metastatic sarcoma. Cancer Treat Rep 62:1513-1516, 1978.
12. TEFT M, VAWTER GF, and MITUS A. Second primary neoplasms in children. AJR 103:800-822, 1968.
13. VOLL AR, and TVEIT J. Levkemi hos tidligere roentgen/radium-behandlete pasienter. Nord Med 58:1114-1117, 1957.

Phase I–II Diaziquone Chemotherapy in Brain Tumors[1]

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A quinone-containing alkylating agent, diaziquone (AZQ), was originally designated to cross the blood-brain barrier while retaining antitumor activity (1). The drug has been undergoing clinical trials and has demonstrated some activity against brain tumors (2,3). This study evaluates the activity of AZQ given either intravenously or intra-arterially in patients with primary or metastatic brain tumors.

MATERIALS AND METHODS

Five patients with histologically confirmed primary brain tumors and 17 patients with metastatic brain tumors were treated with AZQ. All patients were evaluated by neurologic examination, electroencephalogram, and computerized tomography scan. Phenytoin, phenobarbital, and dexamethasone were administered to control seizure activity and brain edema.

Eleven patients received 10–12 mg/m^2 of AZQ intravenously daily × 4 every 2–3 weeks. The starting dose of intra-arterial AZQ was 10 mg/m^2 by intracarotid and/or vertebral infusion repeated every 2–3 weeks. After the safety of low-dose AZQ was demonstrated in five patients, the subsequent doses were escalated in individual patients to 20–23 mg/m^2 (four patients) and to 28–30 mg/m^2 (two).

RESULTS

Characteristics of the patient population are shown in table 1. Two partial responses were seen among the eight evaluable patients treated intra-arterially. One of the two patients with partial response who had bilateral malignant melanoma brain metastases received 10 mg/m^2 of AZQ through the right vertebral artery. He showed a 90% decrease of the right occipital tumor after two courses, with neurological improvement of his balance and vision. The other patient achieving partial response who had bronchogenic carcinoma received a total AZQ dose of 20 mg/m^2 through the right internal carotid and

TABLE 1.—Patient characteristics*

	Intra-arterial	Intravenous
Total entered	11	11
Evaluable for toxicity	11	11
Evaluable for response (> 2 courses)	8	8
Prior systemic chemotherapy	11	11
Prior whole-brain irradiation	3	3
Sex		
Female	4	5
Male	7	6
Age range in yrs	40–73	26–73
Performance status		
0	0	0
1	2	0
2	2	3
3	3	8
4	4	0
Primary brain tumor	3	2
Metastatic brain tumor	8	9
Response		
Complete	0	0
Partial	2†	0
Toxicity		
Wbc count nadir (cells/mm^3)		
3000–4000	1	7
2000–2999	1	3
< 2000	2	1
Platelet count nadir (cells/mm^3)		
75,000–100,000	1	3
50,000–74,999	1	2
< 50,000	1	1
Nausea and vomiting	0	11
Neurologic	0	0

* Unless otherwise indicated, values = No. of patients
†6 and 3 mos.

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left vertebral artery twice. He showed a > 50% decrease of all bilateral brain metastases, with normalization of his speech as well as motor function. Responses lasted 6 and 3 months, respectively.

Toxicity

Ten patients who received AZQ intravenously experienced grade 1-2 hematologic toxic effects, and one had leukopenia with a wbc count nadir of 1500/mm^3.

Two patients who received one course of therapy at AZQ doses of 30 and 23 mg/m^2 intra-arterially experienced a drop in the wbc count to 1900 and 1500/mm^3 and in the platelet count to 53,000 and 49,000/mm^3, respectively.

DISCUSSION

Although the number of patients treated in our study was small, these preliminary results might be suggestive of some intra-arterial AZQ activity against metastatic brain tumors. Its value against primary brain tumors could not be properly evaluated. The hematologic toxic effect is the major dose-limiting factor. The recommended schedule of AZQ given intra-arterially is 20 mg/m^2 every 3 weeks, with the total dose not exceeding 40 mg/course.

REFERENCES

1. CHOU F, KAHN AH, and DRISCOLL JS. Potential central nervous system antitumor agents, aziridinyl-benzoquinones 2. J Med Chem 19:1302-1308, 1976.
2. CURT GA, SCHILSKY R, KELLY J, ET AL. Phase II study of aziridinylbenzoquinone (AZQ) in high grade gliomas. Proc ASCO 1:13, 1982.
3. ARONEY RS, KAPLAN RS, SALEMAN M, ET AL. A phase II trial of AZQ in patients with recurrent primary or metastatic brain tumor. Proc ASCO 1:24, 1982.

Prospidin Chemotherapy in Recurrent Head and Neck Carcinoma: A Phase II Study[1]

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Prospidin is a new alkylating cytostatic agent developed in the USSR. It has been shown to be effective against poorly differentiated squamous cell carcinoma (especially the head and neck region), lymphomas, and Kaposi's sarcoma. Exophytic and metastatic tumors are the most sensitive to its action (1-3). In addition to its alkylating activity, prospidin inhibits protein synthesis of many enzymes and hormones, inhibits glycolysis, and is anti-inflammatory (4). It has been well-tolerated. Only mild gastrointestinal disturbances, vertigo, paresthesias, heightened sensitivity to cold, and mild anorexia have been described. Characteristically, hematological side effects have been very uncommon. In this report, we describe the effects of prospidin alone on 23 evaluable patients with previously treated locally recurrent or metastatic head and neck carcinoma.

MATERIALS AND METHODS

Since 1979, 23 adult patients have been included in this phase II study. There were 15 patients with recurrent laryngeal carcinoma after primary treatment with surgery and/or chemotherapy and radiotherapy in one case; three with advanced lingual carcinoma; one with advanced nasopharyngeal lymphoepithelioma; one each with advanced lip, oropharyngeal, and hypopharyngeal carcinoma; and one with cylindroma. The histology of the recurrent tumor was documented by biopsy in each case. Patient and treatment characteristics are shown in table 1.

Prospidin was administered iv in 15 daily doses of 120 mg/m^2 in most patients. In three patients, the treatment time was shortened to 10 days by elevating the daily prospidin dose to 180 mg/m^2. The cycles of the treatment were repeated every 5-6 weeks.

All patients had measurable tumor. Response was evaluated after every cycle of treatment, according to recommendations of the International Union Against Cancer (UICC), the World Health Organization (WHO), and the National Cancer Institute (5). If signs of progressive disease occurred, the patient was referred to other treatment. In case of stable disease or positive objective response, the treatment was continued until remission or disease progression. Toxicity of prospidin treatment was evaluated by measuring ECG, liver enzymes, creatinine, blood cell count, and subjective symptoms of the patient.

RESULTS

Among the 15 evaluable patients with larynx cancer, there were four partial responses of 1, 7, 8, and 11 months' duration. Moreover, three patients had a clear stagnation of the tumor growth (2, 5, and 8 months). Seven patients showed progressive disease, three of them dying from progressive tumor growth during the first cycle of treatment. The three patients with lingual cancer had progressive disease. In the patient with lip cancer and pulmonary metastases and in the patients with lymphoepithelioma, oropharyngeal, and hypopharyngeal carcinoma, the local tumor remained stable for 8, 3, 3, and 7 months, respectively. Prospidin also had a stationary effect of 10 months on the pulmonary metastases of the adenoid cystic carcinoma. The treatment of this patient had to be discontinued because of severe pulmonary fibrosis. Three patients had previously received radiotherapy on the site of recurrent tumor. One of the responders had been treated with cyclophosphamide, vincristine, and bleomycin before prospidin therapy. No correlation could be found between the histopathological malignancy grade and the response. The number of prospidin cycles is shown in table 1.

At the ordinary daily dose of 120 mg/m^2, prospidin therapy was usually well-tolerated and side effects were uncommon. One of the patients had mild leukopenia (2.0 × 10^6 cells/ml), two had fatigue, one had vertigo, and four had mild anorexia. No cardiotoxicity, liver toxicity,

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TABLE 1.—Patient and treatment characteristics*

Patient No.	Age (yrs)	Sex	Diagnosis	Site of recurrent tumor	Previous therapy	No. of cycles and daily dose (mg/m²) of prospidin	Response	Duration of response (mos)	Toxic effects
1	72	M	Laryngeal carcinoma	Local	OP + RT	2 × 120	PD	—	None
2	79	M	Laryngeal carcinoma	Local	RT	5 × 120	PR	11	None
3	52	M	Laryngeal carcinoma	Local	OP + RT	4 × 120	PR	7	Mild anorexia
4	39	M	Laryngeal carcinoma	Local	OP + RT	2 × 120	PR	1	Mild anorexia
5	69	M	Laryngeal carcinoma	Local	OP + RT	2 × 120	PD	—	None
6	65	M	Laryngeal carcinoma	Lung	OP + RT + CT	5 × 120	PR	8	Fatigue
7	68	M	Laryngeal carcinoma	Lung	OP + RT	2 × 120	PD	—	None
8	63	M	Laryngeal carcinoma	Local	RT	4 × 120	NC	8	Vertigo
9	49	M	Laryngeal carcinoma	Lymph node	OP + RT	2 × 120	NC	2	Mild anorexia
10	48	F	Laryngeal carcinoma	Lymph node	RT	1 × 120	PD	—	None
11	61	M	Laryngeal carcinoma	Local	OP + RT	1 × 120	PD	—	None
12	62	F	Laryngeal carcinoma	Local	OP + RT	1 × 120	PD	—	None
13	71	M	Laryngeal carcinoma	Local	OP + RT	1 × 120	PD	—	None
14	79	M	Laryngeal carcinoma	Local	OP + RT	1 × 180	PD	—	Severe myelotoxicity and skin eruption
15	71	M	Laryngeal carcinoma	Local	OP + RT	1 × 180	NC	5	Severe myelotoxicity and skin eruption
16	47	F	Lingual carcinoma	Lung	RT	3 × 120	PD	—	Fatigue
17	38	M	Lingual carcinoma	Lung	RT + CT	1 × 120	PD	—	None
18	64	M	Lingual carcinoma	Lung	RT + CT	1 × 120	PD	—	Mild anorexia
19	70	M	Lip cancer	Lung	OP + RT	4 × 120	NC	8	Mild leukopenia
20	56	M	Oropharyngeal carcinoma	Local	RT	2 × 120	NC	3	None
21	66	F	Hypopharyngeal carcinoma	Local	OP + RT	3 × 120	NC	7	None
22	56	M	Nasopharyngeal lymphoepithelioma	Lymph node	OP + RT	3 × 120	NC	3	None
23	53	F	Cylindroma	Lung	OP	6 × 120 + 1 × 180	NC	10	Pulmonary fibrosis

* OP = surgery; RT = radiotherapy; CT = chemotherapy; PD = progressive disease; PR = partial response; and NC = no change.

or nephrotoxicity was noticed. None of the patients had nausea or vomiting.

Three patients temporarily received a higher dose of prospidin (180 mg/m^2) for 10 consecutive days. After termination of these courses of treatment, two of the patients had severe leukopenia with leukocyte values of 0.3 and 0.5 × 10^6 cells/ml and thrombocytopenia with values of 30 and 50 × 10^6 cells/ml. Both patients had psoriasis-like skin lesions all over the body and ulcerations of the mucosa. These patients needed intensive care and thrombocyte and leukocyte transfusions. Bone marrow was not examined. Blood cell counts returned to normal in 2 weeks, but skin eruptions lasted for 1 month. The patient with cylindroma also receiving a high dose of prospidin had severe interstitial pneumonitis followed by pulmonary fibrosis, both resistant to prednisone and anti-inflammatory drugs. This kind of serious acute toxicity has not been described earlier.

DISCUSSION

We have described a phase II study of 23 evaluable patients with advanced head and neck carcinoma treated with a new alkylating cytostatic drug, prospidin. This piperazine derivative seems to be marginally effective against metastatic squamous cell carcinoma of the larynx. No complete responses were seen, but four of 15 patients had partial responses lasting from 1 to 11 months. Furthermore, three patients had stable disease for 2–8 months. The responders had either locally advanced tumors in previously irradiated areas or untreated metastases. Interestingly, prospidin obviously had no effect on other squamous cell tumors.

With the recommended dose of prospidin (120 mg/m^2/day for 15 consecutive days), toxic effects were almost absent. However, severe hematological, dermatological, and pulmonary toxic effects were seen in three patients with the higher dose of 180 mg/m^2. This was surprising, because animal experiments have shown the LD_{50} for mice to be as high as 250 mg/kg (4).

There is no report of combination chemotherapy with prospidin in head and neck carcinoma. Our preliminary results are so promising that we have a further plan of combining it with cisplatin and bleomycin. With this combination, the course of prospidin treatment should be shortened to 10 days. Preoperative treatment of head and neck carcinoma with prospidin alone should be of interest, because it may have some anti-inflammatory effect and, thus, the operability of the tumor is not affected by mucosal reactions caused by local radiotherapy or the commonly used bleomycin chemotherapy.

REFERENCES

1. DOSYCHEV EA, and PAVLOV J. Regional perfusions with prospidin in the treatment of Kaposi's angiosarcoma. Vestn Dermatol Venerol 8:62-64, 1977.
2. MURAUSKAIA G, SERZHANIN I, NISENBAUM L, ET AL. Chemo-radiotherapy of malignant tumors. Vopr Onkol 25:106-110, 1979.
3. POGOSOV V, CHERNOV A, and ANTONIV V. Administration of prospidin in the treatment of patients with cancer of the larynx. Herald of Otorhinolaryngology 1:57-61, 1970.
4. MOSHOVSKY M, SOROKINA Y, CHERNOV V, ET AL. Antioncotic activity of prospidin. Probl Oncol 16:83-85, 1970.
5. WORLD HEALTH ORGANIZATION. WHO Handbook for Reporting Results of Cancer Treatment. Geneva, 1979.

Phase II Clinical Trial of Mitoxantrone in Patients With Advanced Renal Cell Carcinoma: A Southwest Oncology Group Study[1,2]

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Mitoxantrone (DHAD) is a new anthracenedione derivative that was synthesized in an attempt to find an agent with the same or better antitumor activity than that of doxorubicin, without the dose-limiting cardiotoxicity (1,2). Phase I studies of DHAD have shown it to have tolerable and reversible toxicity in humans. The dose-limiting toxic effects have been thrombocytopenia and leukopenia (3). A phase II study of DHAD in advanced renal cell carcinoma is the subject of this report.

MATERIALS AND METHODS

Patient eligibility included histologic confirmation of renal cell carcinoma not amenable to curative surgical therapy; measurable disease; life expectancy of at least 6 weeks; Karnofsky performance status of, at worst, 50-60; wbc count $\geq 3500/mm^3$; platelet count $\geq 125,000/mm^3$; serum creatinine ≤ 2.0 mg/100 ml; and serum bilirubin ≤ 2.0 mg/100 ml. There were no age restrictions. Patients were required to have recovered from the acute toxic effects of any previous therapy. Concomitant radiation therapy was allowed for CNS metastases only. No other concomitant therapy was allowed.

After giving informed consent, patients were treated with 12 mg/m^2 of DHAD given as an iv infusion in 100 ml of 5% dextrose in water over 30 minutes. Drug administration was repeated every 3 weeks as long as disease stabilization or improvement was seen. Patients were removed from the study if objective disease progression occurred following one or more courses of DHAD.

During treatment, patients had a weekly cbc with differential and platelet count. Prior to each course, ECG, urinalysis, tumor measurements, and liver and renal function tests were done. A requirement for ejection fractions before each course was added later in the study.

Complete remission was defined as the complete disappearance of all clinical evidence of active tumor for a minimum of 4 weeks. Partial remission was defined as a $\geq 50\%$ decrease in the sum of the products of two diameters of all measured lesions, with no new disease or increase in size of any lesion. Disease progression was defined as the unequivocal increase of at least 50% in the size of any measurable lesion, the appearance of new lesions, or uncontrolled hypercalcemia.

RESULTS

Thirty-one patients previously untreated with chemotherapy and 22 treated patients were entered in the study. Four patients were not evaluable. There were three early deaths. Forty-nine patients were evaluable for response. The pretreatment characteristics are shown in table 1. There were 153 courses of therapy

TABLE 1.—Pretreatment characteristics

Characteristic	No. of patients (%)
Karnofsky performance status	
90-100	8 (16)
80-89	4 (8)
70-79	13 (26)
60-69	3 (6)
50-59	16 (32)
Not noted	5 (10)
Median age in yrs (range): 57 (37-77)	
Prior treatment	
Radiation therapy	16 (33)
Surgery	27 (55)
Chemotherapy	21 (42)
Infarction of primary tumor	4 (8)
Sites of disease involvement	
CNS	8 (16)
Pulmonary	36 (72)
Liver	9 (18)
Intra-abdominal	12 (24)
Soft tissue	18 (36)
Hypercalcemia	3 (6)
Other	7 (14)

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TABLE 2.—Hematologic toxicity*

	No. of courses by toxicity grade					
	0	1	2	3	4	5
Leukopenia†	21	23	30	17	1	1
Thrombocytopenia‡	86	1	3	1		

* All courses of 12 mg/m^2 were evaluable for toxicity.

†Toxicity grade (wbcs/mm^3): 0 = > 4000; 1 = 3000-4000; 2 = 2000-3000; 3 = 1000-2000; 4 = < 1000; and 5 = treatment-related death.

‡Toxicity grade (platelets/mm^3): 0 = > 100,000; 1 = 75,000-100,000; 2 = 50,000-75,000; 3 = 25,000-50,000; 4 = < 25,000; and 5 = treatment-related death.

given. The median number of courses per patient was two (range, one to 12).

There were no objective responses. Fifteen patients had stable disease lasting from 42 to 259 days. Patients with stable disease had the same distribution of organ involvement and prior treatment as those with progressive disease. The 95% confidence limits for the true proportion of patients responding is $P \leq 0.08$. If the true response rate for this agent was 15%, there is a < 0.1% chance that no responders would have been observed in the group of 49 patients studied.

Toxicity

Thirty-nine patients were evaluable for toxicity. Eleven patients had insufficient data for toxicity evaluation. The major toxic effect was myelosuppression, primarily leukopenia. The median wbc count nadir was 2900/μl (range, 800 to > 4000). The median platelet count nadir was 254,000/μl (range, 42,000 to > 150,000). Table 2 shows the number of courses by toxicity grade. Myelosuppression was not more severe in those patients who had had prior treatment. One patient receiving concomitant radiotherapy to CNS metastases had prolonged thrombocytopenia. There was one treatment-related death because of leukopenia (granulocyte count, 100/mm^3) and pseudomonas sepsis. Six patients had a drop of 2-3 g in hemoglobin.

Mild to moderate nausea and vomiting (eight patients), mild alopecia (three), fatigue (four), mucositis (one), and lightheadedness (one) were the only nonhematologic toxic effects. There was no cardiotoxicity observed clinically or with ECG monitoring. Five patients were monitored with ejection fractions.

DISCUSSION

This study included patients with a good performance status, more than half of whom had received no prior chemotherapy. Although toxicity was seen, including grade 3 and 4 myelosuppression in 20% of the courses evaluated, no major responses were observed. We conclude that DHAD given at this dose and schedule is ineffective in the treatment of patients with advanced renal cell carcinoma.

REFERENCES

1. ZEE-CHENG RKY, and CHENG CC. Antineoplastic agents. Structure activity relationship study of bis (substituted aminoalkylamine) anthraquinones. J Med Chem 21:291-294, 1979.
2. JOHNSON RK, ZEE-CHENG RK-Y, LEE WW, ET AL. Experimental antitumor activity of aminoanthraquinones. Cancer Treat Rep 63:425-439, 1979.
3. VON HOFF DD, POLLARD E, KUHM J, ET AL. Phase I clinical investigation of 1,4-dihydroxy-5,8-bis [[2-2-([hydroxyethyl) amino] ethyl] amino 9,10-anthracenedione dyhydrochloride] (NSC 301739, DHAD) a new anthracenedione. Cancer Res 40:1516-1518, 1980.

Cyclophosphamide, Methotrexate, and 5-FU (CMF)-Induced Nausea and Vomiting: A Controlled Study With High-Dose Metoclopramide[1]

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Nausea and vomiting are among the most frequent and debilitating side effects of cancer chemotherapy. In patients receiving the combination of cyclophosphamide, methotrexate, and 5-FU (CMF), a regimen extensively used as adjuvant chemotherapy for breast cancer, the incidence of these side effects ranges from 60% to 80% (1-3).

Attempts to control the digestive toxicity of CMF, including the usual dose of metoclopramide, have been unsuccessful (2,3). However, some studies with metoclopramide have showed that high doses of this drug are effective in preventing nausea and vomiting induced by a large range of chemotherapeutic agents and combinations, including those which are refractory to conventional antiemetic treatments (4-6). Previous experience with two doses of 1 mg/kg of metoclopramide, one given before and the other given immediately after delivery of the antineoplastic agents, showed that this schedule was efficient and safe enough to be given on an outpatient basis (4). Thus, this schedule was retained to test its efficacy against CMF-induced nausea and vomiting.

PATIENTS AND METHODS

From March to July 1983, 40 patients were included in the study. All patients were women, with a median age of 54 years (range, 35-73) and an OMS general status score of 1 or 2. All patients had undergone a mastectomy for breast carcinoma. None of the patients had previously received antineoplastic agents, and all were eligible for adjuvant chemotherapy.

The chemotherapy regimen consisted of a combination of cyclophosphamide (600 mg/m^2), methotrexate (40 mg/m^2), and 5-FU (600 mg/m^2) given successively by iv injections. The entire course of treatment was completed in 1 hour.

Before receiving their first CMF course, patients were randomly assigned to receive either metoclopramide or a placebo. Metoclopramide (Delagrange Laboratories, Paris) was supplied in 1-ml vials containing 20 mg of the drug; identical vials containing only the diluent were also supplied to be used as the source of the placebo. Metoclopramide (1 mg/kg) or an identical volume of placebo was diluted in 50 ml of 0.9% sodium chloride solution and was infused over 15 minutes, 30 minutes before initiating chemotherapy and immediately after. The total dose for each patient receiving metoclopramide was 2 mg/kg. Neither the patients nor the physicians and nurses administering the antiemetic agents were aware of whether metoclopramide or the placebo was delivered. No other antiemetic agents were given before or during the 24 hours following the treatment.

All patients remained in the hospital for 24 hours after the administration of chemotherapy. Incidences of nausea or vomiting were recorded, as well as the number of episodes of emesis, the duration of each episode, and the duration of nausea. The assessment of the digestive disturbances was accomplished by a physician who otherwise did not participate in the care of the patients. The toxicity scale used was as follows: 0 = no nausea and vomiting; 1 = nausea of < 3 hours' duration or an episode of emesis; 2 = nausea for > 3 hours or two to five episodes of vomiting; and 3 = more than five episodes of vomiting. All of the side effects were recorded, including mainly extrapyramidal reactions, sedation, and diarrhea.

RESULTS

Efficacy

All of the patients completed the chemotherapy course and the antiemetic treatment and were evaluable. Five patients (25%) who received metoclopramide had no nausea or vomiting, three (15%) had only nausea for ≤ 3 hours, and 12 (60%) had marked digestive

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toxicity with more than five episodes of emesis. Among the patients who received placebo, six (30%) had no nausea or vomiting, two (10%) experienced only mild nausea for < 3 hours, two (10%) had two to five episodes of emesis, and ten (50%) had a score of 3 on the toxicity scale. The average episode of vomiting was 5.9 in the metoclopramide-treated group and 5.4 in the placebo-treated group. Considering nausea, the number of episodes of vomiting, and their duration, no difference appeared between the patients who received metoclopramide and those who received placebo. Furthermore, the median latency time of nausea and vomiting was 9 hours (range, 6-18) for both groups.

Tolerance

In the metoclopramide group, 11 patients presented with symptoms of sedation, which were mild in all but one patient. Diarrhea was recorded in nine patients and was severe in five. An extrapyramidal syndrome occurred in two patients (trismus, one; akathisia, one), but in both instances, it was rapidly reversed by an im injection of trihexyphenidyl. Three patients also experienced headache. Among the placebo-treated patients, symptoms of mild sedation were noted in two and mild diarrhea was noted in five.

DISCUSSION

In this study, high doses of metoclopramide failed to prevent or decrease nausea and vomiting, which remain the most frequent side effects of CMF adjuvant chemotherapy for breast cancer. The modifications introduced in the treatment schedule reducing the treatment period from 15 days every 4 weeks (1) to 1 day every 3 weeks were designed to reduce the contingencies related to the treatment. According to our experience, the modifications have also already been successful in reducing the hematological side effects and in reducing dramatically the incidence of alopecia. However, as shown in this study, significant nausea and vomiting still occur in 60% of the treated patients.

With CMF delivered according to this 1-day schedule, nausea and vomiting turn out to be late side effects, occurring after a 6-12-hour latency and prolongated by vomiting during 24-48 hours. This can be compared with the digestive side effects of high-dose cyclophosphamide used alone (7), which may be due to the time required by the hepatic metabolism of the drug towards cytotoxic and, possibly, emetic compounds. If nausea and vomiting are in fact due to cyclophosphamide, the failure of the high-dose metoclopramide regimen used in this study can be explained by the rapid metabolic clearance of this drug, whose half-life ranges from 3.9 to 5.3 hours (8). The hope that this rapid elimination could be offset in using high-dose metoclopramide was not realized.

We conclude that other attempts to control CMF-induced nausea and vomiting should be performed with other drugs and/or other schedules designed to fit the long latency of their occurrence.

REFERENCES

1. BLOCK W, PINGOUD A, KHAN M, ET AL. The pharmacokinetics, bioequivalence and bioavailability of metoclopramide in man. Arzneim Forsch Drug Res 31:1041-1045, 1981.
2. MORRAN C, SMITH DC, and ANDERSON DA. Incidence of nausea and vomiting with cytotoxic chemotherapy. A prospective randomized trial of antiemetics. Br Med J 1:1323-1324, 1979.
3. WILCOX PM, FETTING JH, NETTESHEIM KM, ET AL. Anticipatory vomiting in women receiving cyclophosphamide, methotrexate, and 5-FU (CMF) adjuvant chemotherapy for breast carcinoma. Cancer Treat Rep 66:1601-1604, 1982.
4. BUI NB, MARIT G, and HOERNI B. High-dose metoclopramide in cancer chemotherapy-induced nausea and vomiting. Cancer Treat Rep 66:2107-2108, 1982.
5. GRALLA RJ, ITRI LM, PISKO SE, ET AL. Antiemetic efficacy of high-dose metoclopramide: randomized trials with placebo and prochlorperazine in patients with chemotherapy induced nausea and vomiting. N Engl J Med 305:905-909, 1981.
6. STRUM SB, MCDERMED JE, OPFELL RW, ET AL. Intravenous metoclopramide: an effective antiemetic in cancer chemotherapy. JAMA 247:2683-2686, 1982.
7. FETTING JH, GROCHOW LB, FOLSTEIN MF, ET AL. The course of nausea and vomiting after high-dose cyclophosphamide. Cancer Treat Rep 66:1487-1493, 1982.
8. BONADONNA G, BRUSAMOLINO E, VALAGUSSA P, ET AL. Combination chemotherapy as an adjuvant treatment in operable breast cancer. N Engl J Med 294:405-410, 1976.

Phase II Trial of Amsacrine in Patients With Hepatoma: A Cancer and Leukemia Group B Study[1]

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Amsacrine (*m*-AMSA), synthesized by Cain and Atwell (1), was selected in 1977 for human studies because of encouraging activity against animal tumors. In phase I trials the dose-limiting toxic effect was myelosuppression (2,3). In 1979, the Cancer and Leukemia Group B initiated this phase II trial of *m*-AMSA in patients with hepatoma, because animal studies showed that high levels of the drug accumulate in the liver (4).

PATIENTS AND METHODS

Patient Selection

Between June 1979 and April 1980, 20 patients were entered in this study of *m*-AMSA in hepatoma. All patients were evaluable (table 1). Patients were required to have measurable, metastatic, histologically documented hepatoma; wbc count $> 4000/\mu l$; platelet count $> 100,000/\mu l$; bilirubin < 3.0 mg/dl; and SGOT less than three times normal.

Drug Administration

m-AMSA was initially administered at a dose of 60–120 mg/m^2, depending on the extent of prior chemotherapy and radiotherapy and abnormal liver function tests. Treatments were given every 3 weeks, and doses were increased by 20 mg/m^2 with each treatment until toxicity was encountered or until a level of 160 mg/m^2 was achieved.

Criteria for Response

Disease was measured by palpable masses, isotope scans, and radiographic studies. Standard response criteria were used (2).

RESULTS

Response

Of the 20 patients treated, one (5%) had a partial response lasting 1 month and four (20%) had stable disease lasting 3, 4, 4, and 5 months (table 2). All other patients had progressive disease. The responding patient and three of the four patients with stable disease had had no prior treatment. The fourth patient who had stable disease for 4 months had received a combination of 5-FU, doxorubicin, and mitomycin. The patient with partial re-

TABLE 1.—*m*-AMSA in hepatoma: pretreatment patient characteristics

Characteristic	No. of patients (%)
Evaluable	20
Male:female	17:3
Median age in yrs (range): 63.5 (20–76)	
Performance status	
Normal or minor impairment	11 (55)
Bedridden < 50% of time	8 (40)
Bedridden > 50% but < 100% of time	0
Bedridden 100% of time	1 (5)
Extent of metastatic disease	
Lung	5 (25)
Nodes	3 (15)
Bone	3 (15)
Spleen	1 (5)
Soft tissue	1 (5)
Prior therapy	
None	13 (65)
Surgery	3 (15)
Chemotherapy	7 (35)
Single-drug	3 (15)
Combination of drugs	4 (20)
Hormonal therapy	1 (5)
Radiotherapy	1 (5)

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TABLE 2.—*m*-AMSA in hepatoma: results

	No. of patients (%)
Toxicity	
Wbc count nadir (cells/μl)	
2000–3000	3 (15)
1000–2000	1 (5)
< 1000	2 (10)
Platelet count nadir (cells/μl)	
20,000–50,000	1 (5)
< 20,000	0
Degree of nausea and vomiting	
None	17 (85)
Mild	1 (5)
Moderate	2 (10)
Severe	0
m-AMSA administered (mg/m^2)	
Initial dose	
60	12 (60)
100	1 (5)
120	7 (35)
Dose ever received	
120	9 (45)
140	5 (25)
160	2 (10)
Initial bilirubin levels (mg/dl)	
0.4–1.3	14 (70)
1.4–1.9	4 (20)
2.0–2.3	2 (10)
Response	
Partial	1 (5)
Stable disease	4 (20)
Disease progression	15 (75)

sponse and two patients with stable disease received *m*-AMSA at 120 mg/m^2. Two patients with stable disease for 4 and 5 months received maximum *m*-AMSA doses of 60 and 80 mg/m^2, respectively.

Drug Toxicity

All 20 patients were evaluable for drug toxicity. Clearly, the dose-limiting toxic effect was myelosuppression (table 2). There was no renal or cardiac toxicity.

DISCUSSION

This phase II study shows that *m*-AMSA is minimally active in hepatoma. Three other recently published phase II trials in hepatoma have shown remarkably similar results, with a partial response rate of only 5% (four of 74 patients) (5–7). Myelosuppression was more severe in these other trials probably because of the high number (60%) of our patients starting at a reduced dose (60 mg/m^2) due to prior treatment or abnormal liver function tests. However, 45% of our patients did receive doses ⩾ 120 mg/m^2 at some time during their treatment, making it unlikely that significant activity was masked by our method of dose selection and escalation.

REFERENCES

1. CAIN BF, and ATWELL GJ. The experimental antitumor properties of three congeners of the acridylmethanesulphonanilide (AMSA) series. Eur J Cancer 10:539–549, 1974.
2. LEGHA SS, GUTTERMAN JU, HALL SW, ET AL. Phase I clinical investigation of 4′-(9-acridinylamino)methanesulfon-*m*-anisidide (NSC 249992), a new acridine derivative. Cancer Res 38:3712–3716, 1978.
3. VON HOFF DD, HOWSER D, GORMLEY P, ET AL. Phase I study of methanesulfonamide, *N*-[4-(9-acridinylamino)-3-methoxyphenyl]-(*m*-AMSA) using a single-dose schedule. Cancer Treat Rep 62:1421–1426, 1978.
4. CYSYK RL, SHOEMAKER DD, AYERS OC, ET AL. Oral absorption and selective tissue localization of 4′-(9-acridinylamino)-methanesulfon-*m*-anisidide. Pharmacology 16:206–213, 1978.
5. FALKSON G, COETZER B, and KLAASSEN DJ. A phase II study of *m*-AMSA in patients with primary liver cancer. Cancer Chemother Pharmacol 6:127–129, 1981.
6. BURKOWSKI RM, LEGHA S, SAIKI J, ET AL. Phase II trial of *m*-AMSA in hepatocellular carcinoma: a Southwest Oncology Group study. Cancer Treat Rep 66:1651–1652, 1982.
7. CHENG E, LIGHTDALE C, YOUNG C, ET AL. Phase II trial of (*m*-AMSA) 4′-9-(acridinylamino)methanesulfon-*m*-aniside in primary liver cancer. Am J Clin Oncol 6:211–213, 1983.

Phase II Study of Continuous 5-Day Vinblastine Infusion in Renal Adenocarcinoma[1,2]

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No reproducibly effective therapy for disseminated renal carcinoma has been found (1). Interest in vinblastine was initially stimulated by a reviewed response rate of 25% (2). Subsequent studies have not confirmed these results (3).

It has been reported that the effectiveness of vinca alkaloids is improved by administration as a continuous infusion rather than by intermittent therapy (4,5). The present study was designed to evaluate a continuous vinblastine infusion schedule in patients with metastatic renal cell carcinoma.

Nineteen patients fulfilled the following criteria for this phase II trial: pathologic (18 patients) or arteriographic (one patient) confirmation of renal adenocarcinoma; measurable or evaluable advanced disease; Eastern Cooperative Oncology Group (ECOG) performance status ≤ 3; no chemotherapy within the previous 3 weeks; adequate renal and hepatic functions; and adequate marrow reserve (wbc count $\geq 4000/mm^3$, platelet count $\geq 100{,}000/mm^3$).

Treatment consisted of vinblastine (Eli Lilly and Co) at an initial dose of 1.5 mg/m²/day in 1000 ml of 5% dextrose in water given as a continuous 24-hour infusion for 5 days. This regimen was repeated approximately every 4 weeks. Patients were continued in the study until disease progression was noted. Hematologic toxicity or other toxic effects in a given treatment cycle dictated a scheduled dose reduction during the subsequent cycle. Provision was also made for 25% dosage escalation (maximum dose, 1.9 mg/m²/day) when no toxicity was recorded in the previous cycle. Ten patients eventually received the higher dosage. Objective tumor responses were classified using standard ECOG criteria.

Patient characteristics and drug toxicity are shown in table 1. There were three objective partial responses (16%). In two patients, pulmonary nodules were decreased after two cycles of therapy. In a third patient, documented shrinkage of liver metastasis occurred after the eighth infusion. These tumor responses have lasted for 10+, 5, and 8 months, respectively.

Two patients had no change in their disease during therapy for 6 and 12 months. Fourteen (74%) patients had disease progression, seven before receiving the second cycle of therapy. There was no difference between responders and patients with early disease progression in terms of performance status, length of time since initial diagnosis, or prior therapy. Two patients who had previously failed to respond to intermittent vinblastine did not respond to continuous infusions. In the current study, life-threatening myelosuppression and severe neurotoxicity were rare. Peripheral phlebitis was a frequent problem and the use of central venous catheters should be considered for future vinblastine infusion studies.

The three objective tumor responses observed in this study suggest a low but definite antineoplastic activity for vinblastine against renal carcinoma (90% confidence interval, 4%–36%). However, continuous infusion of this drug at doses < 2 mg/m²/day does not appear to offer a significant improvement over its use on an intermittent schedule.

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TABLE 1.—Patient characteristics and drug toxicity

	No. of patients
Entered/evaluable	19/19
Male/female	14/5
Median age in yrs (range): 60 (21–79)	
ECOG performance status	
0–2	18
3	1
Weight loss ≥ 10%	3
Prior treatment*	
Renal artery embolization	2 (1 preoperatively)
Nephrectomy	14
Irradiation to primary disease site	1 (postoperatively)
Irradiation to bone metastasis	5
Hormonal therapy	5
Chemotherapy (2 with vinblastine)	5
Interferon	1
Metastasis disease sites	
Lung	12
Abdomen	5
Bone	6
Liver	2
Skin	2
Hematologic toxicity†	
Wbc count nadir (cells/mm^3)	
> 2000	13
1001–2000	1
≤ 1000	1
Platelet count nadir (cells/mm^3)	
> 100,000	15
≤ 100,000	0
Other toxic effects	
Phlebitis	7
Constipation	6
Paresthesias	5
Nausea and vomiting	4
Myalgias and arthralgias	2
Urinary retention‡ (after 12 infusions)	1

*Some patients received > 1 form of therapy.
†Data available on 15 of 19 patients.
‡Resolved within 2–3 weeks after therapy was discontinued.

REFERENCES

1. PAULSON DR, PEREZ CA, and ANDERSON T. Genito-urinary malignancies. Renal adenocarcinoma. *In* Cancer, Principles and Practice of Oncology (DeVita VT, Jr, Hellman S, and Rosenberg SA, eds). Philadelphia, Lippincott, 1982, pp 740–742.
2. HRUSHESKY WJ, and MURPHY CP. Current status of the therapy of advanced renal carcinoma. J Surg Oncol 9:277–288, 1977.
3. HAHN RG, TEMKIN NR, SAVLOV ED, ET AL. Phase II study of vinblastine, methyl-CCNU, and medroxyprogesterone in advanced renal cell cancer. J Surg Oncol 62:1093–1095, 1978.
4. JACKSON DV, and BENDER RA. Cytotoxic thresholds of vincristine in a murine and a human leukemia cell line in vitro. Cancer Res 39:4346–4349, 1979.
5. YAP H-Y, BLUMENSCHEIN GR, KEATING MJ, ET AL. Vinblastine given as a continuous 5 day infusion in the treatment of refractory advanced breast cancer. Cancer 64:279–283, 1980.

Phase II Clinical Trial With Bisantrene in Patients With Advanced Refractory Small Cell Lung Cancer[1,2]

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In a prior study utilizing the anthracene derivative, bisantrene, predictable toxic effects as well as antitumor activity were noted using a single-dose intermittent schedule (1). Concurrent with that phase I study, bisantrene was found to have in vitro activity against small cell carcinoma of the lung in the human tumor cloning system (2). This in vitro activity encouraged an early phase II study in patients with refractory small cell carcinoma of the lung.

PATIENTS AND METHODS

Patients with measurable, histologically documented small cell lung cancer refractory to higher priority therapy (usually vincristine, doxorubicin, and cyclophosphamide alternating with methotrexate, vincristine, and etoposide) were eligible. A performance status of 3 or better (Southwest Oncology Group) and adequate marrow, hepatic, and renal functions were required. Bisantrene, at an initial dose of 260 mg/m^2, was infused iv every 3 weeks. Lack of toxicity permitted dose escalation (to 280 mg/m^2) on subsequent infusions.

Treatment characteristics of the 15 patients in this study are summarized in table 1. Notably, all patients were symptomatic and had had extensive cytotoxic drug and radiation therapy exposure; most had liver metastases.

RESULTS

None of the patients achieved a response. One patient had disease stabilization for 9 weeks prior to disease progression with bisantrene therapy. Dose escalation to 280 mg/m^2 was attempted in the second cycle in three patients without obvious benefit. Overall survival was extremely short from the first day of therapy (median, 4 weeks; range, 1-33).

TABLE 1.—Treatment characteristics of 15 patients

Characteristic	No. of patients
Total	15
Sex	
Male	12
Female	3
Median age in yrs (range): 60 (35-70)	
Median performance status (SWOG) (range): 2 (1-3)	
Sites of indicator lesions	
Liver	9
Lung	8
Nodal	3
Bone	3
Bone marrow	2
Dermal	1
Prior therapy	
Cytotoxic drugs; median (range): 5 (3-6)	
Anthracycline	14
Radiotherapy	15
Courses of bisantrene	
1	8
2	6
3	0
4	1

Toxicity

The short-term survival of this group precludes the reporting of hematologic data in seven patients. Reversible leukopenia (median polymorphonuclear neutrophil count, 1500/mm^3; range, 110-5000), with median nadir on Day 8, was the most common hematologic toxic effect. Although thrombocytopenia was mild, as in other trials, two patients had platelet count nadirs of 49,000 and 22,000/mm^3 without bleeding sequelae. One patient had an anaphylactoid reaction consisting of hypotension, wheezing, and shortness of breath. Other toxic effects in-

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cluded diarrhea (one patient), fever (one), and nausea (one).

DISCUSSION

No activity could be demonstrated for bisantrene in the treatment of refractory small cell carcinoma of the lung when used in this intermittent schedule of every 3 weeks. Unfortunately, this refractoriness may be a generalized phenomenon of small cell lung cancer to a number of phase II drugs after exposure to aggressive front-line therapy. Conceivably, activity would be demonstrable in patients with better performance status, less prior therapy, and more favorable sites of metastatic disease.

REFERENCES

1. VON HOFF DD, MYERS JW, KUHN J, ET AL. Phase I clinical investigation of 9-10 anthracenedicarboxaldehyde bis ((4,5-dihydro-1-*H*-imidazol-2 yl) hydrazone) dihydrochloride (CL216,942). Cancer Res 41:3118-3121, 1981.
2. VON HOFF DD, COLTMAN CA, JR, and FORSETH B. Activity of 9-10 anthracenedicarboxaldehyde bis ((4,5-dihydro-1 *H*-imidazole-2-yl) hydrazone) dihydrochloride (CL216,942) in a human tumor cloning system, leads for phase II trials in man. Cancer Chemother Pharmacol 6:141-144, 1981.

Phase II Evaluation of Amsacrine in Colorectal, Gastric, and Pancreatic Carcinomas: A Southeastern Cancer Study Group Trial[1,2,3]

George A. Omura,* Alfred A. Bartolucci, Howard E. Lessner, and George J. Hill[2]

Amsacrine is an acridine derivative which is active in acute leukemia. Single-institution trials in colorectal (1), gastric (2), and pancreatic (3) cancers have been negative. Our experience with these cancers has been similar.

Patients with biopsy-proven, incurable carcinomas were eligible after written informed consent. At least one easily measurable lesion and a Karnofsky performance status > 50% were required. Amsacrine in 5% dextrose and water was administered by a 1-hour iv infusion at 90 mg/m^2 every 3 weeks. Re-treatment was to be delayed week by week if the total granulocyte count remained < 2500/mm^3 or if the platelet count remained < 100,000/mm^3. If the granulocyte count nadir was < 1000/mm^3 or if the platelet count nadir was < 50,000/mm^3, the next dose was to be decreased to 50%; subsequently, increases to 75% and then to 100% were indicated if re-treatment was tolerated. An adequate trial was defined as two courses of therapy unless there was rapid disease progression. A complete response was defined as the disappearance of all disease; a partial response (PR) was defined as a reduction of at least 50% in the sum of the product of perpendicular diameters in all lesions. Toxic effects were graded according to standard Southeastern Cancer Study Group criteria.

Of 246 patients entered, six were not eligible, seven had major protocol violations, 33 were not adequately treated, and one was evaluable for toxicity only; there were 199 evaluable patients, including 152 with colorectal, 27 with pancreatic, and 20 with gastric cancers. Seventy patients (46%) with colorectal cancer, 20 (47%) with pancreatic cancer, and ten (50%) with gastric cancer had had no prior chemotherapy. Males comprised 79 (52%), 20 (74%), and 13 (65%) of the patients, respectively. The median performance status was 80%, 70%, and 70%, respectively. The median age for patients with colorectal and gastric cancers was 61 years; for patients with pancreatic cancer, the median age was 60 years.

There were only three responders, all with colorectal cancer: one complete response (liver) lasted 1½ months; one PR (subcutaneous mass) lasted 6½ months; and one PR (groin mass) lasted 1¾ months. The complete responder had had no prior chemotherapy but the partial responders had.

Granulocyte count depression to 1500–1800/mm^3 occurred in 9% of the patients, to 1000–1400/mm^3 in 12%, to 500–900/mm^3 in 26%, and to < 500/mm^3 in 26%. Platelet count depression to 90,000–129,000/mm^3 occurred in 9% of the patients, to 50,000–89,000/mm^3 in 9%, to 25,000–49,000/mm^3 in 3%, and to < 25,000/mm^3 in 5%. Other toxic effects are summarized in table 1. Three patients experienced transient "cardiac arrest" during the first, second, and third doses, respectively; all had marked liver dysfunction from cancer at the time of treatment. One of these patients may have had a vasovagal syncope rather than arrest, and one was shown at autopsy to have had an acute myocardial infarction.

The lack of more than occasional responses in the present trial was disappointing but is consistent with previous reports (1–3). With regard to cardiac toxicity, others (4) have reported occasional ventricular arrhythmias associated with amsacrine therapy and have recommended careful attention to serum potassium levels

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[3]Amsacrine lactate was supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

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TABLE 1.—Nonhematologic toxic effects

Toxic effect	No. of patients (%) with grade* —			
	1+	2+	3+	4+
Cardiac				3 (1.5)
Oral	6 (3)			
Gastrointestinal	59 (30)	13 (7)	2 (1)	
Genitourinary	6 (3)	1		
Liver	2 (1)	3 (1.5)		
CNS	8 (4)	3 (1.5)	1	
Other†	9 (5)	8 (4)	2 (1)	

*Grades according to Southeastern Cancer Study Group criteria.
†Includes fever, malaise, myalgia, edema, chills, and fatigue.

during treatment. In summary , the dose schedule we employed is not effective in colorectal, gastric, or pancreatic cancer.

REFERENCES

1. VALDIVIESO M, STROEHLEIN JR, BEDIKIAN AY, ET AL. Phase II clinical evaluation of intravenous 4′-(9-acridinylamino) methanesulfon-*m*-anisidide in colorectal cancer. Am J Clin Oncol 6:39–44, 1983.
2. SORDILLO PP, MAGILL GB, GRALLA RJ, ET AL. Phase II evaluation of 4′-(9-acridinylamino)-methanesulfon-*m*-anisidide (*m*-AMSA) in patients with gastric adenocarcinoma. Am J Clin Oncol 5:273–275, 1982.
3. STERNBERG CN, MAGILL GB, SORDILLO PP, ET AL. Phase II evaluation of *m*-AMSA (4′-(9-acridinylamino)-methanesulfon-*m*-anisidide) in patients with adenocarcinoma of the pancreas. Am J Clin Oncol 6:459–462, 1983.
4. VON HOFF DD, ELSON D, POLK G, ET AL. Acute ventricular fibrillation and death during infusion of 4′-(9-acridinylamino)methanesulfon-*m*-anisidide (AMSA). Cancer Treat Rep 64:356–357, 1980.

Intra-abdominal Carcinomatosis With Histologically Normal Ovaries[1]

At times, oncologists are faced with the patient who presents with ascites positive for malignant cells and no obvious primary site of carcinoma. Robert et al (1) have recently reviewed the approach to the problem of cancers of unknown origin and have emphasized the importance of diagnosing potentially curable tumors, including ovarian carcinoma. Specifically in this setting, they suggest laparotomy to examine the ovaries, even if a pelvic mass is not detected. An intriguing variation on this theme was recently reported by Tobacman et al (2). They followed 28 women from 16 ovarian cancer-prone families after prophylactic oophorectomy. Three of these women developed malignancies which presented in all cases with abdominal masses, peritoneal carcinomatosis, and papillary adenocarcinoma, "indistinguishable histopathologically from ovarian carcinoma." They suggest that at least in such families, the coelomic analage of the ovary may give rise to primary peritoneal tumors.

We would like to report our experience in three patients who had abdominal carcinomatosis with histologically normal ovaries. None of the women had a family history of ovarian carcinoma. All three patients presented with pelvic masses, peritoneal carcinomatosis, and papillary histology of the adenocarcinoma. Exploration and further evaluation did not disclose another primary source. Specifically, the pancreas was judged to be normal. The pathologists considered the primary source of the tumor to be unknown in all three patients. Two patients were treated with a chemotherapy regimen used in ovarian carcinoma and achieved complete response.

CASE REPORTS

Patient 1

In December 1981, a 72-year-old nulliparous (one abortion) white woman presented with 3 months of abdominal swelling and a tender right groin mass. Family history of cancer was specifically denied. Computerized tomographic (CT) scan showed a pelvic mass, one small area consistent with liver metastasis, diffuse peritoneal involvement, and multiple small bowel implants. Barium contrast studies of the upper gastrointestinal tract were normal. Biopsy of the right groin node disclosed "moderately differentiated cystadenocarcinoma" suspected to be of ovarian origin. At exploratory laparotomy, there were ascites, an 8-cm pelvic mass, diffuse peritoneal involvement, and a mesenteric cyst. The uterus, tubes, and both ovaries were identified to be totally free of tumor. The pancreas was therefore suggested as a possible primary site. Nevertheless, the patient was treated for presumptive ovarian carcinoma with cisplatin (20 mg/m^2) for 5 days and cyclophosphamide (600 mg/m^2) on Day 4 (3). She received five successful courses of this regimen and two further courses of cyclophosphamide alone (because of cisplatin-induced paresthesias). She continues to do well > 1 year later. Repeat CT scan 10 months after surgery showed objective decrease in intra-abdominal and hepatic disease; a subsequent CT scan was entirely normal. She is currently without evidence of disease 18 months after diagnosis.

Patient 2

In May 1982, a nulliparous 58-year-old white female presented with malodorous vaginal discharge. A pelvic mass was present and vaginal cytology was positive. Colonoscopy suggested the presence of a fistula. At laparotomy, she had a 14-cm tumor involving the left parametrial area and sigmoid colon, as well as several other smaller peritoneal masses. Pathology of these masses showed mucin-positive adenocarcinoma and the same appearance as the tumors in the omentum, sigmoid colon serosa, and left fallopian tube. The ovaries were totally unremarkable. Family history of ovarian cancer was denied. The patient was treated with the same regimen as Patient 1 and achieved a clinical remission. At the present date, re-evaluation indicates no evidence of disease.

Patient 3

In January 1981, a 59-year-old mother of four children presented with pain and tenderness over a right groin mass. In 1962, she had had a left oophorectomy because of a left ovarian serous cyst and, in 1971, a vaginal hysterectomy had been performed for menometrorrhagia. On biopsy of the right inguinal mass, a poorly differentiated adenocarcinoma, partially papillary, was noted. An exploratory laparotomy, omentectomy, left salpingectomy, right salpingo-oophorectomy, and pelvic lymph node dissection were performed. Papillary adenocarcinoma was found metastatic to eight of ten lymph nodes and the right pelvic side wall. Surprisingly, the re-

[1]Supported in part by American Cancer Society Institutional Cancer Research Fund IN-14Y.

maining ovary and tubes were normal. After further evaluation, including thyroid scans and mammography, she was considered for treatment of a carcinoma, primary unknown. She received 3740 rads of whole-pelvic irradiation and was then started on a regimen of chlorambucil (6 mg/day) and megestrol (160 mg/day). This was discontinued 6 months later when left axillary node metastases were discovered; 4600 rads to the axilla were given. In August 1982, she complained of left radicular leg pain and CT scan suggested involvement of sacral plexus; 3000 additional rads were given to this area without effective palliation. She died shortly thereafter, 20 months after the initial laparotomy. An autopsy was not performed.

We conclude that intra-abdominal carcinomatosis which clinically and pathologically resembles ovarian carcinoma may arise with histologically normal ovaries, as well as in women who have undergone prophylactic oophorectomy because of a high-risk family history. Furthermore, we have had success treating two of these patients with chemotherapy known to be effective against ovarian carcinoma (4,5). Our experience further suggests the existence of a peritoneal malignancy which biologically, clinically, and perhaps developmentally resembles ovarian carcinoma. It may also respond to therapy similar to that used for ovarian carcinoma. This consideration is particularly important in light of the excellent results in treating even advanced ovarian disease with cyclophosphamide and cisplatin (5).

REFERENCES

1. ROBERT NJ, GARNICK MB, and FREI E. Cancers of unknown origin: current approaches and future perspectives. Semin Oncol 9:526-531, 1982.
2. TOBACMAN JK, TUCKER MA, KASE R, ET AL. Intra-abdominal carcinomatosis after prophylactic oophorectomy in ovarian cancer prone families. Lancet 2:795-797, 1982.
3. YOUNG RC, KNAPP RC, and PEREZ CA. Cancer of the ovary. *In* Cancer Principles and Practice of Oncology (DeVita VT, Jr, Hellman S, and Rosenberg SA, eds). Philadelphia, J. B. Lippincott Co, 1982, p 902.
4. WERNZ JC, SPEYER JL, NOUMOFF HJ, ET AL. Cisplatin/cytoxan: a high dose DDP regimen for advanced stage ovarian carcinoma. Proc ASCO 1:112, 1982.
5. DECKER DG, FLEMING TR, MALKASIAN GD, JR, ET AL. Cyclophosphamide plus cisplatinum in combination: treatment program for stage III or IV ovarian carcinoma. Obstet Gynecol 60:481-487, 1982.

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Palliative Treatment of Head and Neck Squamous Cancer With Cisplatin and Hydroxyurea

Both hydroxyurea and cisplatin have demonstrated activity against squamous cell carcinomas of the head and neck. Hydroxyurea has produced seven responses (only one qualifying as a > 50% regression) in 18 evaluable patients (1,2), and cisplatin has demonstrated a response rate of approximately 33% independent of dosage and duration of infusion in several studies in patients with advanced head and neck cancer following failure of local therapy (3). Cisplatin and hydroxyurea have demonstrated synergism in various animal models, especially when hydroxyurea is given in large doses prior to cisplatin (4). Speculation exists that hydroxyurea might cause cell cycle block in S phase, with subsequent increased sensitivity to cisplatin as a mechanism for this synergism (5). In support of hydroxyurea being an active drug in combination with other agents in patients with head and neck cancer, the combination of bleomycin, methotrexate, and hydroxyurea produced 21 responses, including 11 complete responses in 32 such patients for 10 months in one study (6), and the combination of bleomycin and hydroxyurea produced nine responses in 19 such patients in a second study (7). These response rates are considerably higher than those anticipated from methotrexate and bleomycin without hydroxyurea. Studies of the combination of bleomycin and methotrexate have generally produced partial responses in < 50% of patients with head and neck cancer (8), and those with bleomycin alone have reported a 30% response rate in equivalent patients (9).

With these considerations, we initiated a study of hydroxyurea plus cisplatin in patients with squamous cancer of the head and neck who had recurrence of disease following the failure of prior local therapy, including radiation therapy, or who had distant metastases. Hydroxyurea (1500 mg/m^2) was given orally 4 hours before cisplatin (60 mg/m^2), which was given as an iv infusion on Day 1. Hydroxyurea (1500 mg/m^2/day) was then given in divided doses on Days 8-21. The cycle was repeated every 3 weeks and the dosage was adjusted based on blood cell count suppression.

Of the first eight patients treated, seven had received prior radiation therapy; five of these seven also had had prior surgery. One patient presenting with distant metastases had received no prior treatment. Only two patients had received prior chemotherapy (methotrexate alone and methotrexate plus vindesine, respectively). Five of the patients were female and three were male. Ages ranged from 42 to 61 years and all patients had a Zubrod performance status of 2 or better. Time from initial diagnosis to protocol therapy ranged from < 1 to

50 months (median, 20). Three patients had local disease only, four had nodal disease, and two had distant metastases.

No patient had a tumor reduction sufficient to qualify as a partial response, although two showed transient improvement. All patients had disease progression between 2 and 12 weeks of starting therapy. Survival ranged from 21 to 43 weeks (median, 34).

Toxicity consisted of leukopenia (four patients with < 2000 cells/mm^3), thrombocytopenia (one patient with < 50,000 cells/mm^3), and nausea and vomiting. No nephrotoxicity was observed.

We would anticipate at least a 30% response rate to cisplatin alone in this group of patients. The cisplatin dose of 60 mg/m^2 every 3 weeks used in this study is comparable to that used in other drug combinations reporting response rates in > 50% of patients (6). To suggest any benefit from the addition of hydroxyurea to cisplatin, an increase in the response rate of at least 10% over that expected from cisplatin alone would be required. The absence of any responses in eight consecutive patients excludes a 40% response rate to the drug combination with > 95% confidence. Because of the low probability of this schedule of cisplatin and hydroxyurea being synergistic, the study was discontinued.

REFERENCES

1. BLOEDOW CE. Phase II studies of hydroxyurea (NSC-32065) in adults: miscellaneous tumors. Cancer Chemother Rep 40:39-41, 1964.
2. LERNER HJ, BECKLOFF GL, and GODWIN MC. Hydroxyurea (NSC-32065) intermittent therapy in malignant diseases. Cancer Chemother Rep 53:385-395, 1969.
3. TAYLOR SG, IV. Head and neck cancer. *In* Cancer Chemotherapy, the EORTC Cancer Chemotherapy Annual 1 (Pinedo HM, ed). Amsterdam, Excerpta Medica, 1979, vol 1, pp 237-256.
4. GALE GR, ATKINS LM, MEISCHEN SJ, ET AL. Schedule-dependency assessments of ribonucleoside disphosphate reductase inhibitors when used in combination with platinum compounds plus cyclophosphamide in the treatment of advanced L1210 leukemia. Cancer Treat Rep 63:449-456, 1979.
5. TOMITA K, and PLAGER JE. In vivo cell cycle synchronization of the murine Sarcoma 180 tumor following alternating periods of hydroxyurea blockade and release. Cancer Res 39:4407-4411, 1979.
6. MEDENICA R, ALBERTO P, and LEHMANN W. Combined chemotherapy of head and neck squamous cell carcinomas with methotrexate, bleomycin and hydroxyurea. Cancer Chemother Pharmacol 5:145, 1981.
7. EISENBERGER MA, DENEFRIO J, SILVERMAN M, ET AL. Combination chemotherapy with bleomycin and hydroxyurea in the treatment of advanced head and neck cancer. Cancer Treat Rep 66:1439-1440, 1982.
8. TAYLOR SG, IV. Head and neck cancer. *In* Cancer Chemotherapy, the EORTC Cancer Chemotherapy Annual 5 (Pinedo HM, and Chabner BA, eds). Amsterdam, Excerpta Medica, 1983, vol 5, pp 291-306.
9. CARTER SK. The chemotherapy of head and neck cancer. Semin Oncol 4:413-424, 1977.

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Increase of Serum Iron Concentrations in Rhesus Monkeys and Humans on Administration of Vincristine, Vinblastine, or Vindesine[1]

The antitumor alkaloids vincristine and vinblastine from the *Catharanthus* species are widely used as single agents or in combination with other chemotherapeutic agents against a variety of neoplastic diseases. A chemically modified derivative of vinblastine, vindesine, known to be less neurotoxic in experimental animals, is undergoing phase II clinical trials (1). At clinical iv bolus doses of these alkaloids, there is a rapid disappearance of the alkaloids from serum, and at 4-6 hours after injection, serum alkaloid concentrations in the range of 10^{-8} to 10^{-9} M in humans (2) and rhesus monkeys (3) have been obtained. The percent of the alkaloid in body serum compartment is < 0.5% of the injected dose at 4 hours after injection (3), and the total urinary and fecal excretion of the alkaloid over a 5-day period is in the range of 20%-30% (3). Here we report the increase of serum iron concentration in rhesus monkeys and cancer patients on administration of these alkaloids.

[1]Supported in part by Public Health Service grant CA-12197 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

Chemical analysis of daily serum samples from two rhesus monkeys after injection of vincristine indicated that concentrations of Na^+, K^+, BUN, glucose, albumin, total bilirubin, alkaline phosphatase, and creatinine did not change. Serum LDH activity showed a 2.5-2.7-fold increase over the pretreatment values, and these levels returned to preinjection values within 2-3 days (table 1). Similarly, SGOT activity was also increased by 1.7-2.2-fold, and these activities returned to preinjection levels within 4-7 days. Serum iron concentrations exhibited maximum increase of 3.3-4.4-fold over the pretreatment levels, and these values also returned to preinjection levels within 4-7 days (table 1). When consecutive weekly injections of vincristine were administered to the rhesus monkeys, serum iron concentrations were elevated within 2-3 days to sixfold over the preinjection values (fig 1A). These concentrations fell to normal levels within 7 days. After administration of vinblastine (0.20 mg/kg) or vindesine (0.10 mg/kg), a similar stimulation of serum iron concentrations was observed (fig 1B).

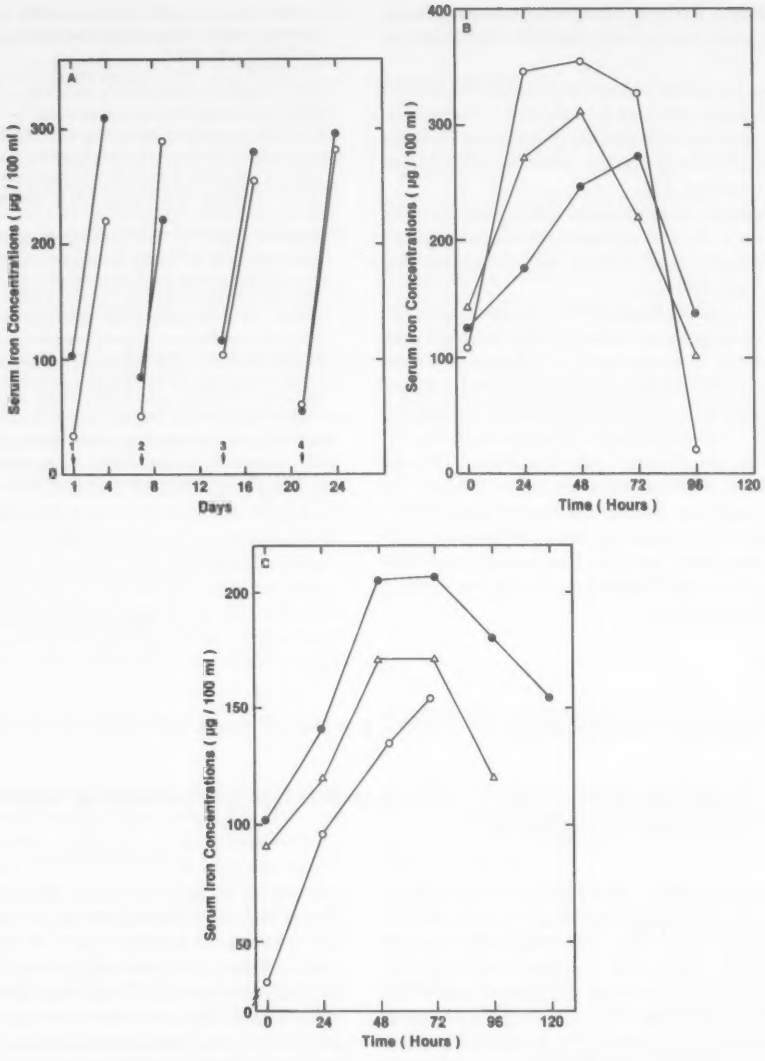
A
Serum Iron Concentrations (µg / 100 ml)
Days
B
Serum Iron Concentrations (µg / 100 ml)
Time (Hours)
C
Serum Iron Concentrations (µg / 100 ml)
Time (Hours)

FIGURE 1.—A: Elevation of serum iron concentration in rhesus monkeys after 4 consecutive weekly iv bolus injections of vincristine (0.15 mg/kg). O——O = Monkey 199, and ●——● = Monkey 722. B: Kinetics of the increase of serum iron concentration in rhesus monkeys after iv bolus administration of the *Catharanthus* alkaloids. O——O = vincristine (0.15 mg/kg) given to Monkey 722, ●——● = vinblastine (0.20 mg/kg) given to Monkey 492, and Δ——Δ = vindesine (0.10 mg/kg) given to Monkey 722. C: Plots of serum iron concentration *vs* time in patients after administration of the alkaloids. O——O = vincristine, ●——● = vinblastine, and Δ——Δ = vindesine. Serum samples beyond 72 hrs for vincristine were not obtained.

Serum from three patients who had undergone therapy with the *Catharanthus* alkaloids was also analyzed for iron concentrations. Characteristics of these patients were as follows: a female with non-Hodgkin's lymphoma (9 years old, 34.1 kg, 137 cm) was given iv bolus vincristine (2.0 mg); a female with acute myelogenous leukemia (46 years old, 63.6 kg, 159 cm) was given a 0.5-mg loading iv bolus dose of vinblastine followed by a continuous infusion of the alkaloid (0.85 mg/m^2 for 5 days); and a female with breast carcinoma with metastasis to lungs (38 years old, 73.6 kg, 161 cm) received an iv bolus dose of vindesine (3.0 mg/m^2). Serum iron concentrations were

TABLE 1.—Chemistry profile analysis (SMAC) of serum samples before and after injection of vincristine in rhesus monkeys*

Monkey No.	Day of SMAC value	Alkaline phosphatase (units/L)	LDH (units/L)	SGOT (units/L)	Iron (μg/dl)
199	0†	103	453	26	81
	1	144	1152	58	321
	2	101	514	52	351
	3	95	277	ND‡	310
	4	105	355	34	153
	7	132	244	27	57
722	0†	93	459	40	109
	1	97	1254	69	348
	2	84	680	68	357
	3	81	459	ND‡	329
	4	90	671	57	120
	7	116	444	36	108

*Male rhesus monkeys (No. 199: 13.3-kg body weight, 12 yrs old; and No. 722: 10.6-kg body weight, 6 yrs old) were free of any pathological symptoms. They were donated by Dr. Thomas B. Clarkson and were fed Purina Monkey Chow 25 (Ralston Purina Co, St. Louis, MO) ad libitum. Animals were injected with 0.15 mg/kg of vincristine in the saphenous vein, and the blood samples were withdrawn from the femoral vein. Samples were centrifuged and resultant sera were analyzed on a Sequential Multiple Analyzer computerized (SMAC) instrument (Technicon Instruments Corp, Tarrytown, NY). The total serum iron concentrations were determined on the SMAC analyzer according to the method of Giovaniello et al (ref 4). The assay was linear up to 800 μg/dl of iron. Values of other serum components which were not affected by the alkaloid were as follows: Na^+ (147–150 mEq/L), K^+ (3.1–4.5 mEq/L), BUN (20–25 mg/dl), glucose (65–99 mg/dl), albumin (3.8–4.2 g/dl), total bilirubin (0.1–0.3 mg/dl), and creatinine (0.9–1.5 mg/dl).

†Vincristine was injected on Day 0 (preinjection values).

‡ND = not determined.

increased 2.5–4.0-fold from the preinjection level by 2–3 days. These levels returned to normal concentrations by 5–7 days (fig 1C). The serum transferrin or ferritin concentrations in the humans or rhesus monkeys after injection of the alkaloids remained unaffected (data not shown).

After administration of vincristine (0.05 mg/kg) or vinblastine (0.2 mg/kg), the rbc count in the rhesus monkeys did not change; the count was between 4.5 and 6.2 × 10^6/μl (data not shown). The hematocrit and the hemoglobin values were also not affected (data not shown). However, there was a stimulation of the wbc count in the first 24 hours from 7.15 ± 1.7 × 10^3/μl to 12.8 ± 1.0 × 10^3/μl for vincristine and from 5.8 ± 0.7 × 10^3/μl to 12.1 ± 1.2 × 10^3/μl for vinblastine. These elevated wbc values returned to preinjection levels within 3–6 days (data not shown).

From this study, mechanism of increase of serum iron concentration by the *Catharanthus* alkaloids cannot be deduced. Since hematocrit, hemoglobin, and serum transferrin and ferritin concentrations did not appreciably change after the alkaloid administration, it could be assumed that the increase in serum iron concentration may be related to inhibition of cellular iron uptake and/or other unknown mechanisms. To our knowledge, this is the first report in the literature on the effect of cytotoxic drugs on iron metabolism.

The increase of serum iron concentrations during treatment with these alkaloids may present a problem in the interpretation of the significance of elevated serum iron concentration in cancer patients. It is well known that subnormal serum iron levels are obtained in iron deficiency anemia, late pregnancy, "anemia of chronic disease" (such as during infection, inflammation, or malignancy), and during acute hemorrhage (5). In contrast, higher serum iron values may be observed in pernicious anemia, hemolytic anemia, acute hepatitis, transfusion hemosiderosis, or primary hemachromatosis (5). Our data would suggest that caution should be exercised in the interpretation of the significance of serum iron levels following chemotherapy with the *Catharanthus* alkaloids.

REFERENCES

1. GERZON K. Dimeric *Catharanthus* alkaloids. *In* Anticancer Agents Based on Natural Product Models (Cassady JM, and Douros JD, eds). New York, Academic Press, 1980, pp 271–317.
2. SETHI VS, JACKSON DV, WHITE DR, ET AL. Pharmacokinetics of vincristine sulfate in adult cancer patients. Cancer Res 41:3551–3555, 1981.
3. SETHI VS, SURRATT P, and SPURR CL. Pharmacokinetics of vincristine, vinblastine and vindesine in rhesus monkeys. Cancer Chemother Pharmacol 12:31–35, 1984.
4. GIOVANIELLO TJ, DI BENEDETTO G, PALMER DN, ET AL. Fully automated method for the determination of serum iron and total iron-binding capacity. *In* Automation in Analytical Chemistry, Technicon Symposia, White Plains, NY, Mediad, Inc., 1976, vol 1, pp 185–188.
5. POLLYCOVE M. Hematochromatosis. *In* The Metabolic Basis of Inherited Disease (Standbury JB, Wyngaarden JB, and Fredrickson DS, eds). New York, McGraw-Hill, 1978, pp 1127–1164.

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Tenth International RES Congress

Ito, Japan, September 2–7, 1984

The scientific program of this meeting, to be held at the Kawana Hotel, will be chaired by Drs. Dolph O. Adams and Ralph Snyderman (Duke University, Durham, NC). Dr. Mizu Kojima (University of Tsukuba, Ibaraki-Ken, Japan) is the President of the Congress and International Host.

The scientific sessions will consist of symposia, mini-symposia, targeted sessions, poster sessions, and a keynote address. Symposia topics include: Viruses, Oncogenes and Human Lymphomas; Macrophages and Atherogenesis; Macrophages as Regulators of Multiple Host Systems; Macrophages and Activation; and Macrophages and Stimulus-Response Coupling.

For further information, contact: Reticuloendothelial Society, c/o Dr. Sherwood M. Reichard, Medical College of Georgia, Augusta, GA 30912 (404-828-2601).

Conference on Immunity to Cancer

Williamsburg, Virginia, September 10–12, 1984

A conference to present recent advances in the various branches of cancer immunology will be held at the Colonial Williamsburg Conference Center. This conference is co-sponsored by the Biological Response Modifiers Program (BRMP) of the National Cancer Institute and the Monsanto Chemical Company. Other sponsors are the Hoffmann-La Roche Co, Eli Lilly and Co, Johnson & Johnson, Inc, New England Nuclear-DuPont, Inc, Bristol-Myers, Inc, ICI-Americas, and McNeil Pharmaceutical. Co-chairmen are Drs. Arnold E. Reif and Malcolm S. Mitchell.

Five sessions of invited papers will be presented entitled: Identification and Characterization of Tumor Antigens; Immune Responses to Tumor Antigens; Regulation of Immune Responses to Tumor Cells; Immunotherapy and Biomodulators; and Immunotherapy and Future Prospects. Participants are invited to submit a one-page abstract, identified by session title, for a Poster Session (deadline: *June 15, 1984*). From the abstracts chosen for the poster presentation, a minority will be selected for inclusion in the Proceedings.

There will be a registration fee of $75 made payable to Program Resources, Inc (deadline: *July 15, 1984*). Early registration is suggested as the conference is limited to 300 participants.

For further information, contact: Ms. Carole Kirby, BRMP, Division of Cancer Treatment, National Cancer Institute, Frederick Cancer Research Facility, Bldg 567, Rm 129, Frederick, MD 21701 (301-695-1418).

Sixth International Symposium on Prevention and Detection of Cancer

Vienna, Austria, November 26–29, 1984

Sponsored by the International Society for Preventive Oncology, World Health Organization, Austrian Cancer Society-Austrian Cancer League, and Association of Clinical Scientists-USA, the program for this symposium includes overview lectures, panels, poster sessions, scientific exhibits, and special workshops designed for critical appraisal of current data. Discussions are to concentrate on actions to be taken and on the implementation of existing knowledge for effective cancer control by primary and secondary prevention. Reports are to present progress in multifactorial etiology of oncogenesis, molecular biology, identification of high-risk groups, tumor susceptibility, and clinical and laboratory manifestations of cancer including tumor markers. Participants include the clinician, epidemiologist, pathologist, experimental oncologist, immunologist, socioeconomist, educator, and members of the health care team.

Abstracts of presentations are invited by June 15, 1984; abstracts and the program will appear in *Cancer Detection and Prevention*, 1984, vol 7, No. 6. Awards for outstanding investigation(s) pre-

sented by ISPO. Accredited for 32 CME credit hours.

For further information, contact: Prevention and Detection of Cancer, AMEX POB #790459, Dallas, TX 75379 [telephone: 1-800-527-0297; outside Europe (usa-214)392-3663); in Europe (austria 43-222)52-0544].

Symposium on Perspectives in Non-Hodgkin's Lymphoma in Children

New Delhi, India, December 3–5, 1984

A panel of international experts will present the latest information on non-Hodgkin's lymphoma in children at this symposium, sponsored by the Indian Cancer Society. Symposium topics will include epidemiology, pathology, immunology, natural history, and management of disease. New guidelines will also be discussed.

For further information, contact: Dr. P. A. Shetty, MD, Department of Medical Oncology, Tata Memorial Hospital, Parel, Bombay 400 012, India.

Fourth World Conference on Lung Cancer

Toronto, Canada, August 25–30, 1985

An anticipated 2500 members of the international medico-scientific community with special interest in lung cancer will gather to share new ideas, research results, and experiences. Under the auspices of the International Association for the Study of Lung Cancer, the conference will be a combination of panels, symposia, lectures, workshops, ciné clinics, and exhibits.

Topics addressed will be prevention, epidemiology, respiratory carcinogenesis, early diagnosis and staging pathology, surgery, radiotherapy, chemotherapy, immunotherapy, cellular kinetics, tumor markers, and imaging. The site of the sessions and exhibit will be the Metro Toronto Convention Center in downtown Toronto.

For further information, contact: Dr. Ronald Feld, Secretary General, The IV World Conference on Lung Cancer, c/o Secretariat Office, 342 MacLaren St, Ottawa, Ontario, Canada K2P OM6.

Third International Congress on Hormones and Cancer

Rome, Italy, April 23–27, 1986

For information contact: Stefano Iacobelli, MD, Laboratorio di Endocrinologia Molecolare, Istituto di Clinica Ostetrica e Ginecologica, Universitá Cattolica del S. Cuore, L.go Agostino Gemelli, 8, 00168-Rome, Italy (telephone: (06) 330.54.496-33.70.551; telex: 611330 UNCATRO).

Cancer Treatment Symposia: Proceedings of the Conference on 2′-Deoxycoformycin: Current Status and Future Directions (Volume 2, 1984)

Sponsored by the Division of Cancer Treatment, National Cancer Institute, and Warner-Lambert/Parke-Davis Pharmaceutical Research Division, this conference was held on April 13, 1983, in Bethesda, MD. Its purpose was to examine the pharmacologic, biochemical, biologic, and clinical information that had accrued in the 4 years of early clinical investigation of 2′-deoxycoformycin (pentostatin; dCF) and to suggest directions for further study.

Initially introduced with the sole purpose of inhibiting the deamination of purine antimetabolites, dCF produces a broad range of biochemical perturbations, with consequences for both normal and malignant cells that are just beginning to be understood. However, the toxic effects of the drug have often been severe and unpredictable and are the basis for the caution with which current trials are progressing. The investigation of toxic effects (description, prediction, and management) was therefore a major focus of this meeting.

Copies of this issue of *Cancer Treatment Symposia* are available for $5.00 from the US Government Printing Office. Use the order form on the last page of this journal.

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